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(54) Title: THE AIOLOS GENE (57) Abstract This invention features an Aiolos gene and polypeptide. The Aiolos polypeptide has a molecular weight of approximately 58 kd and has an N-terminal zinc finger domain. The Aiolos gene is expressed in committed lymphoid progenitors as well as in T and B cells. Therapies for disorders of the immune system, T or B cell related disorders, asthma, or immune mediated skin disorders are presented. Transgenic animals are provided with an Aiolos transgene.		

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THE AIOLOS GENE

Background of the Invention

The invention relates to the Aiolos gene, Aiolos polypeptide, Aiolos homodimers, Aiolos/Ikaros heterodimers and methods of using Aiolos nucleic acids and polypeptides.

Summary of the Invention

In general, the invention features an Aiolos polypeptide, e.g., a polypeptide which includes all or part of the sequence shown in SEQ ID NO:2 or SEQ ID NO:8. The invention also features fragments and analogs of Aiolos polypeptides, preferably having at least one biological activity of an Aiolos polypeptide.

In preferred embodiments, the polypeptide is a recombinant or a substantially pure preparation of an Aiolos polypeptide.

In preferred embodiments, the polypeptide is a vertebrate, e.g., a mammalian, e.g., a human polypeptide.

In preferred embodiments, the Aiolos polypeptide includes additional Aiolos coding sequences 5' to that of SEQ ID NO:8. In preferred embodiments: the additional sequence includes at least 1, 10, 20, 40, 60, 70, 80 or 100 amino acid residues; the additional sequence is equal to or less than 1, 10, 20, 40, 60, 70, 80 or 100 amino acid residues.

In preferred embodiments: the polypeptide has at least one biological activity, e.g., it reacts with an antibody, or antibody fragment, specific for an Aiolos polypeptide; the polypeptide includes an amino acid sequence at least 60%, 80%, 90%, 95%, 98%, or 99% homologous to an amino acid sequence from SEQ ID NO:2 or SEQ ID NO:8; the polypeptide includes an amino acid sequence essentially the same as an amino acid sequence in SEQ ID NO:2 or SEQ ID NO:8; the polypeptide is at least 5, 10, 20, 50, 100, 150, 200, or 250 amino acids in length; the polypeptide includes at least 5, preferably at least 10, more preferably at least 20, most preferably at least 50, 100, 150, 200, or 250 contiguous amino acids from SEQ ID NO:2 or SEQ ID NO:8; the polypeptide is preferably at least 10, but no more than 100, amino acids in length; the Aiolos polypeptide is either, an agonist or an antagonist, of a biological activity of a naturally occurring Aiolos polypeptide.

In preferred embodiments: the Aiolos polypeptide is encoded by the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:7, or by a nucleic acid having at least 60%, 70%, 80%, 90%, 95%, 98%, or 99% homology with the nucleic acid of SEQ ID NO:1 or SEQ ID NO:7. For example, the Aiolos polypeptide can be encoded by a nucleic acid sequence which differs from a nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:7 due to degeneracy in the genetic code.

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In a preferred embodiment, the Aiolos polypeptide encodes amino acid residues 1-507 of SEQ ID NO:2 or a functionally equivalent residue in the Aiolos sequence of another vertebrate or mammal, e.g., a human.

5 In a preferred embodiment, the Aiolos polypeptide encodes amino acid residues 58-507 of SEQ ID NO:2 or a functionally equivalent residue in the Aiolos sequence of another vertebrate or mammal, e.g., a human.

In a preferred embodiment, the Aiolos polypeptide encodes amino acid residues 72-507 of SEQ ID NO:2 or a functionally equivalent residue in the Aiolos sequence of another vertebrate or mammal, e.g., a human.

10 In a preferred embodiment, the Aiolos polypeptide encodes amino acid residues 76-507 of SEQ ID NO:2 or a functionally equivalent residue in the Aiolos sequence of another vertebrate or mammal, e.g., a human.

In a preferred embodiment, the Aiolos polypeptide encodes amino acid residues 1-209 of SEQ ID NO:8.

15 In a preferred embodiment the Aiolos polypeptide is an agonist of a naturally-occurring mutant or wild type Aiolos polypeptide (e.g., a polypeptide having an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:8). In another preferred embodiment, the polypeptide is an antagonist which, for example, inhibits an undesired activity of a naturally-occurring Aiolos polypeptide (e.g., a mutant polypeptide).

20 In a preferred embodiment, the Aiolos polypeptide differs in amino acid sequence at 1, 2, 3, 5, 10 or more residues, from a sequence in SEQ ID NO:2 or SEQ ID NO:8. The differences, however, are such that the Aiolos polypeptide exhibits at least one biological activity of an Aiolos polypeptide, e.g., the Aiolos polypeptide retains a biological activity of a naturally occurring Aiolos polypeptide.

25 In preferred embodiments the Aiolos polypeptide includes an Aiolos polypeptide sequence, as described herein, as well as other N-terminal and/or C-terminal amino acid sequences.

30 In preferred embodiments, the polypeptide includes all or a fragment of an amino acid sequence from SEQ ID NO:2 or SEQ ID NO:8, fused, in reading frame, to additional amino acid residues, preferably to residues encoded by genomic DNA 5' to the genomic DNA which encodes a sequence from SEQ ID NO:2 or SEQ ID NO:8.

35 In yet other preferred embodiments, the Aiolos polypeptide is a recombinant fusion protein having a first Aiolos polypeptide portion and a second polypeptide portion having an amino acid sequence unrelated to an Aiolos polypeptide. The second polypeptide portion can be, e.g., any of glutathione-S-transferase, a DNA binding domain, or a polymerase activating domain. In preferred embodiment the fusion protein can be used in a two-hybrid assay.

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In a preferred embodiment, the Aiolos polypeptide is a fragment or analog of a naturally occurring Aiolos polypeptide which inhibits reactivity with antibodies, or F(ab')₂ fragments, specific for a naturally occurring Aiolos polypeptide.

5 In a preferred embodiment, the Aiolos polypeptide includes a sequence which is not present in the mature protein.

Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and postranslational events.

10 In preferred embodiments, the Aiolos polypeptide: is expressed in spleen and thymus; is expressed in mature T and/or B cells; is highly homologous, preferably at least 90% or 95% homologous, with the 50 most C-terminal amino acids of the Ikaros gene (e.g., the dimerization domain of exon 7 of the Ikaros gene); is highly homologous, preferably at least 90% or 95% homologous with the activation domain of exon 7 of the Ikaros gene; is capable of forming Aiolos dimers and/or Aiolos/Ikaros dimers; is involved in lymphocyte
15 differentiation, e.g., T cell maturation.

In preferred embodiments, the Aiolos polypeptide includes: the YAS5 interaction domain; the YAS3 interaction domain; the YIZ Ikaros dimerization domain.

20 In preferred embodiments, an Aiolos polypeptide encodes: one, two, three, four, five exons, or more exons; exons 3, 4, 5 and 7; exons 3-7; exon 7 (the exons are shown in Fig. 4).

In preferred embodiments, the Aiolos polypeptide has one or more of the following properties:

- (a) it can form a dimer with an Aiolos or Ikaros polypeptide;
- (b) it is expressed in committed lymphoid progenitors;
- 25 (c) it is expressed in committed T and B cells;
- (d) it has a molecular weight of approximately 58 kD;
- (e) it has at least one zinc finger domain;
- (f) it is not expressed in stem cells; or
- (g) it is a transcriptional activator of a lymphoid gene.

30 In other preferred embodiments, the Aiolos polypeptide has one or more of the following properties:

- (a) it can form a dimer with an Aiolos or Ikaros polypeptide;
- (b) it is expressed in committed lymphoid progenitors;
- (c) it is expressed in committed T and B cells;
- 35 (d) it has a molecular weight of approximately 58 kD;
- (e) it has an N-terminal zinc finger domain;
- (f) it is not expressed in stem cells; or
- (g) it is a transcriptional activator of a lymphoid gene.

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In yet other preferred embodiments, the Aiolos polypeptide has one or more of the following properties:

- (a) it can form a dimer with an Aiolos or Ikaros polypeptide;
- (b) it is expressed in committed lymphoid progenitors;
- 5 (c) it is expressed in committed T and B cells;
- (d) it has a molecular weight of approximately 58 kD;
- (e) it has at least one or preferably two C-terminal zinc finger domains;
- (f) it is not expressed in stem cells; or
- (g) it is a transcriptional activator of a lymphoid gene.

10 The invention includes an immunogen which includes an active or inactive Aiolos polypeptide, or an analog or a fragment thereof, in an immunogenic preparation, the immunogen being capable of eliciting an immune response specific for the Aiolos polypeptide, e.g., a humoral response, an antibody response, or a cellular response. In preferred embodiments, the immunogen comprising an antigenic determinant, e.g., a unique
15 determinant, from a protein represented by SEQ ID NO:2 or SEQ ID NO:8. For example, the immunogen comprises amino acids 1-124 of SEQ ID NO:2 or amino acids 275-448 of SEQ ID NO:2.

The invention also includes an antibody preparation, preferably a monoclonal antibody preparation, specifically reactive with an epitope of the Aiolos immunogen or
20 generally of an Aiolos polypeptide.

In another aspect, the invention provides a substantially pure nucleic acid having, or comprising, a nucleotide sequence which encodes a polypeptide, the amino acid sequence of which includes, or is, the sequence of an Aiolos polypeptide, or analog or fragment thereof.

25 In preferred embodiments, the nucleic acid encodes a vertebrate, e.g., a mammalian, e.g., a human polypeptide.

In preferred embodiments, the nucleic acid encodes an Aiolos polypeptide which includes additional Aiolos coding sequences 5' to that SEQ ID NO:8. In preferred
embodiments: the additional sequence includes at least 1, 10, 20, 40, 60, 70, 80 or 100
30 amino acid residues; the additional sequence is equal to or less than 1, 10, 20, 40, 60, 70, 80 or 100 amino acid residues.

In preferred embodiments, the nucleic acid encodes a polypeptide having one or more of the following characteristics: at least one biological activity of an Aiolos, e.g., a polypeptide specifically reactive with an antibody, or antibody fragment, directed against
35 an Aiolos polypeptide; an amino acid sequence at least 60%, 80%, 90%, 95%, 98%, or 99% homologous to an amino acid sequence from SEQ ID NO:2 or SEQ ID NO:8; an amino acid sequence essentially the same as an amino acid sequence in SEQ ID NO:2 or SEQ ID NO:8, the polypeptide is at least 5, 10, 20, 50, 100, 150, 200, or 250 amino acids in

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length; at least 5, preferably at least 10, more preferably at least 20, most preferably at least 50, 100, 150, 200, or 250 contiguous amino acids from SEQ ID NO:2 or SEQ ID NO:8; an amino acid sequence which is preferably at least 10, but no more than 100, amino acids in length; the ability to act as an agonist or an antagonist of a biological activity of a naturally occurring Aiolos polypeptide.

In preferred embodiments: the nucleic acid is or includes the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:7; the nucleic acid is at least 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous with a nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:7; the nucleic acid includes a fragment of SEQ ID NO:1 or SEQ ID NO:7 which is at least 25, 50, 100, 200, 300, 400, 500, or 1,000 bases in length; the nucleic acid differs from the nucleotide sequence of SEQ ID NO:1 due to degeneracy in the genetic code.

In a preferred embodiment, the Aiolos encoding nucleic acid sequence encodes amino acid residues 1-507 of SEQ ID NO:2 or a functionally equivalent residue in the Aiolos sequence of another vertebrate or mammal, e.g., a human.

In a preferred embodiment, the Aiolos encoding nucleic acid sequence encodes amino acid residues 58-507 of SEQ ID NO:2 or a functionally equivalent residue in the Aiolos sequence of another vertebrate or mammal, e.g., a human.

In a preferred embodiment, the Aiolos encoding nucleic acid sequence encodes amino acid residues 72-507 of SEQ ID NO:2 or a functionally equivalent residue in the Aiolos sequence of another vertebrate or mammal, e.g., a human.

In a preferred embodiment, the Aiolos encoding nucleic acid sequence encodes amino acid residues 76-507 of SEQ ID NO:2 or a functionally equivalent residue in the Aiolos sequence of another vertebrate or mammal, e.g., a human.

In a preferred embodiment, the Aiolos encoding nucleic acid sequence encodes amino acid residues 1-209 of SEQ ID NO:8.

In a preferred embodiment the polypeptide encoded by the nucleic acid is an agonist which, for example, is capable of enhancing an activity of a naturally-occurring mutant or wild type Aiolos polypeptide. In another preferred embodiment, the encoded polypeptide is an antagonist which, for example, inhibits an undesired activity of a naturally-occurring Aiolos polypeptide (e.g., a polypeptide having an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:8).

In a preferred embodiment, the encoded Aiolos polypeptide differs in amino acid sequence at 1, 2, 3, 5, 10 or more residues, from a sequence in SEQ ID NO:2 or SEQ ID NO:8. The differences, however, are such that the encoded Aiolos polypeptide exhibits at least one biological activity of a naturally occurring Aiolos polypeptide (e.g., the Aiolos polypeptide of SEQ ID NO:2 or SEQ ID NO:8).

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In preferred embodiments, the nucleic acid encodes an Aiolos polypeptide which includes an Aiolos polypeptide sequence, as described herein, as well as other N-terminal and/or C-terminal amino acid sequences.

5 In preferred embodiments, the nucleic acid encodes a polypeptide which includes all or a portion of an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:8, fused, in reading frame, to additional amino acid residues, preferably to residues encoded by genomic DNA 5' to the genomic DNA which encodes a sequence from SEQ ID NO:2 or SEQ ID NO:8.

10 In preferred embodiments, the encoded polypeptide is a recombinant fusion protein having a first Aiolos polypeptide portion and a second polypeptide portion having an amino acid sequence unrelated to an Aiolos polypeptide. The second polypeptide portion can be, e.g., any of glutathione-S-transferase; a DNA binding domain; or a polymerase activating domain. In preferred embodiments the fusion protein can be used in a two-hybrid assay.

15 In preferred embodiments, the encoded polypeptide is a fragment or analog of a naturally occurring Aiolos polypeptide which inhibits reactivity with antibodies, or F(ab')₂ fragments, specific for a naturally occurring Aiolos polypeptide.

20 In preferred embodiments, the nucleic acid will include a transcriptional regulatory sequence, e.g. at least one of a transcriptional promoter or transcriptional enhancer sequence, operably linked to the Aiolos gene sequence, e.g., to render the Aiolos gene sequence suitable for use as an expression vector.

25 In yet another preferred embodiment, the nucleic acid of the invention hybridizes under stringent conditions to a nucleic acid probe corresponding to at least 12 consecutive nucleotides from SEQ ID NO:1 or SEQ ID NO:7, or more preferably to at least 20 consecutive nucleotides from SEQ ID NO:1 or SEQ ID NO:7, or more preferably to at least 40 consecutive nucleotides from SEQ ID NO:1 or SEQ ID NO:7.

In a preferred embodiment, the nucleic acid encodes an Aiolos polypeptide which includes a sequence which is not present in the mature protein.

30 In preferred embodiments, the nucleic acid encodes an Aiolos polypeptide which: is expressed in spleen and thymus; is expressed in mature T and/or B cells; is highly homologous, preferably at least 90% or 95% homologous, with the 50 most C-terminal amino acids of the Ikaros gene (e.g., the dimerization domain of exon 7 of the Ikaros gene); is highly homologous, preferably at least 90% or 95% homologous, with the activation domain of exon 7 of the Ikaros gene; is capable of forming Aiolos dimers and/or Aiolos/Ikaros dimers; is involved in lymphocyte differentiation, e.g., T cell maturation.

35 In preferred embodiments, the nucleic acid encodes an Aiolos polypeptide which includes: the YAS5 interaction domain; the YAS3 interaction domain; the YIZ Ikaros dimerization domain.

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In preferred embodiments, the nucleic acid encodes an Aiolos polypeptide which encodes: one, two, three, four, five exons, or more exons; exons 3, 4, 5 and 7; exons 3-7; exon 7 (the exons are shown in Fig. 4).

5 In preferred embodiments, the nucleic acid encodes an Aiolos polypeptide which has one or more of the following properties:

- (a) it can form a dimer with an Aiolos or Ikaros polypeptide;
- (b) it is expressed in committed lymphoid progenitors;
- (c) it is expressed in committed T and B cells;
- (d) it has a molecular weight of approximately 58 kD;
- 10 (e) it has at least one zinc finger domain;
- (f) it is not expressed in stem cells; or
- (g) it is a transcriptional activator of a lymphoid gene.

In other preferred embodiments, the nucleic acid encodes an Aiolos polypeptide which has one or more of the following properties:

- 15 (a) it can form a dimer with an Aiolos or Ikaros polypeptide;
- (b) it is expressed in committed lymphoid progenitors;
- (c) it is expressed in committed T and B cells;
- (d) it has a molecular weight of approximately 58 kD;
- (e) it has an N-terminal zinc finger domain;
- 20 (f) it is not expressed in stem cells; or
- (g) it is a transcriptional activator of a lymphoid gene.

In yet other preferred embodiments, the nucleic acid encodes an Aiolos polypeptide which has one or more of the following properties:

- (a) it can form a dimer with an Aiolos or Ikaros polypeptide;
- 25 (b) it is expressed in committed lymphoid progenitors;
- (c) it is expressed in committed T and B cells;
- (d) it has a molecular weight of approximately 58 kD;
- (e) it has at least one or preferably two C-terminal zinc finger domains;
- (f) it is not expressed in stem cells; or
- 30 (g) it is a transcriptional activator of a lymphoid gene.

In another aspect, the invention includes: a vector including a nucleic acid which encodes an Aiolos polypeptide; a host cell transfected with the vector; and a method of producing a recombinant Aiolos polypeptide, including culturing the cell, e.g., in a cell culture medium, and isolating the Aiolos polypeptide, e.g., an Aiolos polypeptide from the cell or from the cell culture medium.

35 In another aspect, the invention features, a purified recombinant nucleic acid having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homology with a nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:8.

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The invention also provides a probe or primer which includes or comprises a substantially purified oligonucleotide. The oligonucleotide includes a region of nucleotide sequence which hybridizes under stringent conditions to at least 10 consecutive nucleotides of sense or antisense sequence from SEQ ID NO:1 or SEQ ID NO:8, or naturally occurring mutants thereof. In preferred embodiments, the probe or primer further includes a label group attached thereto. The label group can be, e.g., a radioisotope, a fluorescent compound, an enzyme, and/or an enzyme co-factor. Preferably the oligonucleotide is at least 10 and less than 20, 30, 50, 100, or 150 nucleotides in length.

The invention involves nucleic acids, e.g., RNA or DNA, encoding a polypeptide of the invention. This includes double stranded nucleic acids as well as coding and antisense single strands.

The invention includes vertebrate, e.g., mammalian, e.g., rodent, e.g., mouse or rat, or human Aiolos polypeptides.

In another aspect, the invention features a method of evaluating a compound for the ability to interact with, e.g., bind, or modulate, e.g., inhibit or promote, the activity of an Aiolos polypeptide, e.g., an Aiolos monomer, or an Aiolos-Aiolos dimer or an Aiolos-Ikaros dimer. The method includes contacting the compound with the Aiolos polypeptide, and evaluating the ability of the compound to interact with or form a complex with the Aiolos polypeptide. This method can be performed *in vitro*, e.g., in a cell free system, or *in vivo*, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules which interact with the Aiolos polypeptide. It can also be used to find natural or synthetic inhibitors of mutant or wild type Aiolos polypeptide. The compound can be a peptide or a non peptide molecule, e.g., a small molecule preferably 500 to 5,000 molecular weight, more preferably 500 to 1,000 molecular weight, having an aromatic scaffold, e.g., a bis-amide phenol, decorated with various functional groups.

In brief, a two hybrid assay system (see e.g., Bartel et al. (1993) *Cellular Interaction in Development: A practical Approach*, D.A. Hartley, ed., Oxford University Press, Oxford, pp. 153-179) allows for detection of protein-protein interactions in yeast cells. The known protein, e.g., an Aiolos polypeptide, is often referred to as the "bait" protein. The proteins tested for binding to the bait protein are often referred to as "fish" proteins. The "bait" protein, e.g., an Aiolos polypeptide, is fused to the GAL4 DNA binding domain. Potential "fish" proteins are fused to the GAL4 activating domain. If the "bait" protein and a "fish" protein interact, the two GAL4 domains are brought into close proximity, thus rendering the host yeast cell capable of surviving a specific growth selection.

In another aspect, the invention features a method of identifying active fragments or analogs of an Aiolos polypeptide. The method includes first identifying a compound, e.g., an Ikaros peptide, which interacts with an Aiolos polypeptide and determining the ability of

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the compound to bind the candidate fragment or analog. The two hybrid assay described above can be used to obtain fragment-binding compounds. These compounds can then be used as "bait" to fish for and identify fragments of the Aiolos polypeptide which interact, bind, or form a complex with these compounds.

5 In another aspect, the invention features a method of making an Aiolos polypeptide, having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of a naturally occurring Aiolos polypeptide. The method includes altering the sequence of an Aiolos polypeptide (e.g., SEQ ID NO:2 or SEQ ID NO:8) by, for example, substitution or deletion of one or more residues of a non-conserved region, and testing the altered polypeptide for
10 the desired activity.

 In another aspect, the invention features a method of making a fragment or analog of an Aiolos polypeptide, e.g., an Aiolos polypeptide having at least one biological activity of a naturally occurring Aiolos polypeptide. The method includes altering the sequence, e.g., by substitution or deletion of one or more residues, preferably which are non-
15 conserved residues, of an Aiolos polypeptide, and testing the altered polypeptide for the desired activity.

 In another aspect, the invention features, a method of evaluating a compound for the ability to bind a nucleic acid encoding an Aiolos gene regulatory sequence. The method includes: contacting the compound with the nucleic acid; and evaluating ability of the
20 compound to form a complex with the nucleic acid. In preferred embodiments the Aiolos gene regulatory sequence is functionally linked to a heterologous gene, e.g., a reporter gene.

 In another aspect, the invention features a human cell, e.g., a hematopoietic stem cell or a lymphocyte e.g., a T or a B cell, transformed with a nucleic acid which encodes an
25 Aiolos polypeptide.

 In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder, e.g., an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, including administering a therapeutically-effective amount of an Aiolos polypeptide to the
30 animal. The Aiolos polypeptide can be monomeric or an Aiolos-Aiolos or Aiolos-Ikaros dimer.

 In preferred embodiments: the disorder is characterized by unwanted, e.g., higher than normal, antibody, e.g., IgE, production or levels; the disorder is characterized by an antibody mediated response, e.g., an IgE mediated response; the disorder is characterized
35 by an aberrant or unwanted B cell response; the disorder is asthma, an immune mediated skin disorder, e.g., excema, an allergic reaction, hay fever, hives, a food allergy; the disorder is characterized by a hypersensitive response, e.g., an IgE mediated hypersensitive response; the disorder is characterized by an anaphylactic response; the disorder is

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characterized by a local B cell mediated response; the disorder is characterized by a systemic B cell mediated response; the disorder is characterized by unwanted mast cell degranulation.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse. The method includes administering to the animal a cell selected, e.g., selected in vitro, for the expression of a product of the Aiolos gene, e.g., hematopoietic stem cells, e.g., cells transformed with Aiolos-peptide-encoding DNA, e.g., hematopoietic stem cells transformed with Aiolos-peptide-encoding DNA.

In preferred embodiments: the disorder is characterized by unwanted, e.g., higher than normal, antibody, e.g., IgE, production or levels; the disorder is characterized by an antibody mediated response, e.g., an IgE mediated response; the disorder is characterized by an aberrant or unwanted B cell response; the disorder is asthma, an immune mediated skin disorder, e.g., excema, an allergic reaction, hay fever, hives, a food allergy; the disorder is characterized by a hypersensitive response, e.g., an IgE mediated hypersensitive response; the disorder is characterized by an anaphylactic response; the disorder is characterized by a local B cell mediated response; the disorder is characterized by a systemic B cell mediated response; the disorder is characterized by unwanted mast cell degranulation.

In preferred embodiments: the cells are taken from the animal to which they are administered; the cells are taken from an animal which is MHC matched with the animal to which they are administered; the cells are taken from an animal which is syngeneic with the animal to which they are administered; the cells are taken from an animal which is of the same species as is the animal to which they are administered.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse. The method includes administering to the animal a nucleic acid encoding an Aiolos peptide and expressing the nucleic acid.

In preferred embodiments: the disorder is characterized by unwanted, e.g., higher than normal, antibody, e.g., IgE, production or levels; the disorder is characterized by an antibody mediated response, e.g., an IgE mediated response; the disorder is characterized by an aberrant or unwanted B cell response; the disorder is asthma, an immune mediated skin disorder, e.g., excema, an allergic reaction, hay fever, hives, a food allergy; the disorder is characterized by a hypersensitive response, e.g., an IgE mediated hypersensitive response; the disorder is characterized by an anaphylactic response; the disorder is characterized by a local B cell mediated response; the disorder is characterized by a

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systemic B cell mediated response; the disorder is characterized by unwanted mast cell degranulation.

In another aspect, the invention features a method of evaluating the effect of a treatment, e.g., a treatment designed to promote or inhibit hematopoiesis, including
5 carrying out the treatment and evaluating the effect of the treatment on the expression of the Aiolos gene.

In preferred embodiments the treatment is administered: to an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, or a cell, e.g., a cultured
10 stem cell.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Aiolos gene, e.g., a proliferative disorder, e.g., a leukemic disorder, Hodgkin's lymphoma, a cutaneous cell lymphoma, e.g., a cutaneous T cell lymphoma, or a disorder of the immune system, e.g., an
15 immunodeficiency, or a T or B cell related disorder, e.g., a disorder characterized by a shortage of T or B cells. The method includes examining the subject for the expression of the Aiolos gene, non-wild type expression or mis-expression being indicative of risk.

In preferred embodiments: the disorder is characterized by unwanted, e.g., higher than normal, antibody, e.g., IgE, production or levels; the disorder is characterized by an
20 antibody mediated response, e.g., an IgE mediated response; the disorder is characterized by an aberrant or unwanted B cell response; the disorder is asthma, an immune mediated skin disorder, e.g., excema, an allergic reaction, hay fever, hives, a food allergy; the disorder is characterized by a hypersensitive response, e.g., an IgE mediated hypersensitive response; the disorder is characterized by an anaphylactic response; the disorder is
25 characterized by a local B cell mediated response; the disorder is characterized by a systemic B cell mediated response; the disorder is characterized by unwanted mast cell degranulation.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Aiolos gene, e.g., a
30 proliferative disorder, e.g., a leukemic disorder, Hodgkin's lymphoma, a cutaneous cell lymphoma, e.g., a cutaneous T cell lymphoma, or a disorder of the immune system, e.g., an immunodeficiency, or a T or B cell related disorder, e.g., a disorder characterized by a shortage of T or B cells. The method includes providing a nucleic acid sample from the subject and determining if the structure of an Aiolos gene allele of the subject differs from
35 wild type.

In preferred embodiments: the disorder is characterized by unwanted, e.g., higher than normal, antibody, e.g., IgE, production or levels; the disorder is characterized by an antibody mediated response, e.g., an IgE mediated response; the disorder is characterized

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by an aberrant or unwanted B cell response; the disorder is asthma, an immune mediated skin disorder, e.g., excema, an allergic reaction, hay fever, hives, a food allergy; the disorder is characterized by a hypersensitive response, e.g., an IgE mediated hypersensitive response; the disorder is characterized by an anaphylactic response; the disorder is characterized by a local B cell mediated response; the disorder is characterized by a systemic B cell mediated response; the disorder is characterized by unwanted mast cell degranulation.

In preferred embodiments: the determination includes determining if an Aiolos gene allele of the subject has a gross chromosomal rearrangement; the determination includes sequencing the subject's Aiolos gene.

In another aspect, the invention features, a method of evaluating an animal or cell model for a proliferative disorder, e.g., a leukemic disorder. Hodgkin's lymphoma, a cutaneous cell lymphoma, e.g., a cutaneous T cell lymphoma, or an immune disorder, e.g., a T cell related disorder, e.g., a disorder characterized by a shortage of T or B cells. The method includes determining if the Aiolos gene in the animal or cell model is expressed at a predetermined level or if the Aiolos gene is mis-expressed. In preferred embodiments: the predetermined level is lower than the level in a wild type or normal animal; the predetermined level is higher than the level in a wild type or normal animal; or the pattern of isoform expression is altered from wildtype.

In preferred embodiments: the disorder is characterized by unwanted, e.g., higher than normal, antibody, e.g., IgE, production or levels; the disorder is characterized by an antibody mediated response, e.g., an IgE mediated response; the disorder is characterized by an aberrant or unwanted B cell response; the disorder is asthma, an immune mediated skin disorder, e.g., excema, an allergic reaction, hay fever, hives, a food allergy; the disorder is characterized by a hypersensitive response, e.g., an IgE mediated hypersensitive response; the disorder is characterized by an anaphylactic response; the disorder is characterized by a local B cell mediated response; the disorder is characterized by a systemic B cell mediated response; the disorder is characterized by unwanted mast cell degranulation.

In another aspect, the invention features, a transgenic animal, e.g., a mammal, e.g., a mouse or a nonhuman primate having an Aiolos transgene.

In preferred embodiments the animal is a transgenic mouse having a mutated Aiolos transgene, the mutation occurring in, or altering, e.g., a domain of the Aiolos gene described herein.

In other preferred embodiments the transgenic animal or cell: is heterozygous for an Aiolos transgene; homozygous for an Aiolos transgene; includes a first Aiolos transgene

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and a second Aiolos transgene; includes an Aiolos transgene and a second transgene which is other than an Aiolos transgene, e.g., an Ikaros transgene.

In another aspect, the invention features a method for evaluating the effect of a treatment on a transgenic cell or animal having an Aiolos transgene, e.g., the effect of the treatment on the development of the immune system. The method includes administering the treatment to a cell or animal having an Aiolos transgene, and evaluating the effect of the treatment on the cell or animal. The effect can be, e.g., the effect of the treatment on: Aiolos or Ikaros expression or misexpression; the immune system or a component thereof; the nervous system or a component thereof; or the cell cycle. Immune system effects include e.g., T cell activation, T cell development, the ability to mount an immune response, the ability to give rise to a component of the immune system, B cell development, NK cell development, or the ratios $CD4^+/CD8^+$, $CD4^+/CD8^-$ and $CD4^-/CD8^+$.

In preferred embodiments the treatment can include: the administration of a drug, chemical, or other substance; the administration of ionizing radiation; the administration of an antibody, e.g., an antibody directed against a molecule or cell of the immune system; administration of a substance or other treatment which suppresses the immune system; or administration of a substance or other treatment which activates or boosts the function of the immune system; introduction of a nucleic acid, e.g., a nucleic acid which encodes or expresses a gene product, e.g., a component of the immune system; the introduction of a protein, e.g., a protein which is a component of the immune system.

In another aspect, the invention features a method for evaluating the effect of a treatment on an immune system component. The method includes: (1) supplying a transgenic cell or animal having an Aiolos transgene; (2) supplying the immune system component; (3) administering the treatment; and (4) evaluating the effect of the treatment on the immune system component.

In yet another aspect, the invention features a method for evaluating the interaction of a first immune system component with a second immune system component. The method includes: (1) supplying a transgenic cell or animal, e.g., a mammal, having an Aiolos transgene; (2) introducing the first and second immune system component into the transgenic cell or mammal; and (3) evaluating an interaction between the first and second immune system components.

Mice with mutant Aiolos transgenes which eliminate many of the normal components of the immune system, e.g., mice homozygous for a transgene having a deletion for some or all of exon 7 (corresponding to amino acids 275-507 of SEQ ID NO:2), are particularly useful for "reconstitution experiments."

In another aspect, the invention features a method for evaluating the effect of a treatment on an immune system disorder, e.g., a neoplastic disorder, a leukemia or a lymphoma, a T cell related lymphoma, including: administering the treatment to a cell or

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animal having an Aiolos transgene, and evaluating the effect of the treatment on the cell or animal. The effect can be, e.g., the effect of the treatment on: Aiolos or Ikaros expression or misexpression; the immune system or a component thereof; or the cell cycle. Immune system effects include e.g., T cell activation, T cell development, the ability to mount an immune response, the ability to give rise to a component of the immune system, B cell development, NK cell development, or the ratios $CD4^+/CD8^+$, $CD4^+/CD8^-$ and $CD4^-/CD8^+$.

The inventors have also discovered that Ikaros and Aiolos can form dimers (heterodimers) with other polypeptides. E.g., an Ikaros polypeptide can form dimers not only with Ikaros polypeptides, but with other polypeptides which bind to its C terminal region, e.g., other polypeptides having Zinc-finger regions, e.g., Aiolos polypeptides. Similarly, an Aiolos polypeptide can form dimers not only with Aiolos polypeptides, but with other polypeptides which bind to its C terminal region, e.g., other polypeptides having Zinc-finger regions, e.g., Ikaros polypeptides.

The invention also includes Ikaros-Aiolos dimers. The Ikaros member of the dimer can be any Ikaros polypeptide, e.g., any naturally occurring Ikaros or any Ikaros referred to in U.S.S.N.08/238,212, filed May 2, 1994, hereby incorporated by reference. The proteins of the Ikaros family are isoforms which arise from differential splicing of Ikaros gene transcripts. The isoforms of the Ikaros family generally include a common 3' exon (Ikaros exon E7, which includes amino acid residues 283-518 of the mouse Ikaros protein represented by SEQ ID NO:18, and amino acid residues 229-461 of the human Ikaros protein represented by SEQ ID NO:16) but differ in the 5' region. The Ikaros family includes all naturally occurring splicing variants which arise from transcription and processing of the Ikaros gene. Five such isoforms are described herein and in U.S.S.N. 08/238,212, filed May 2, 1994, hereby incorporated by reference. The Ikaros family also includes other isoforms, including those generated by mutagenesis and/or by *in vitro* exon shuffling. The naturally occurring Ikaros proteins can bind and activate (to differing extents) the enhancer of the CD3 δ gene, and are expressed primarily in early hematopoietic and lymphoid cells in the adult. The expression pattern of this transcription factor during embryonic development suggests that Ikaros proteins play a role as a genetic switch regulating entry into the lymphoid and T cell lineages. The Ikaros gene is also expressed in the proximal corpus striatum during early embryogenesis in mice. As is discussed herein, Ikaros and Aiolos polypeptide can form Ikaros-Aiolos dimers.

Accordingly, the invention includes a substantially pure dimer which includes (or consists essentially of) an Aiolos polypeptide and an Ikaros polypeptide.

The Ikaros polypeptide of the Ikaros-Aiolos dimer includes one or more Ikaros exons. In preferred embodiments: the Ikaros exon is E1/2, E3, E4, E5, E6, or E7; the peptide does not include exon E7.

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In other preferred embodiments: the Ikaros peptide of the Ikaros-Aiolos dimer further includes a second Ikaros exon; the second exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7 and the second exon is any of E1/2, E3, E4, E5, E6.

5 In other preferred embodiments: the Ikaros peptide of the Ikaros-Aiolos dimer further includes a third Ikaros exon; the third exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E3, and the third exon is E1/2; the peptide is Ikaros isoform 5.

10 In other preferred embodiments: the Ikaros peptide of the Ikaros-Aiolos dimer further includes a fourth Ikaros exon; the fourth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E4, the third exon is E3, and the fourth exon is E1/2; the first exon is E7, the second exon is E4, the third exon is E3, and the fourth exon is E1/2; the peptide is Ikaros isoform 3 or 4..

15 In other preferred embodiments: the Ikaros peptide of the Ikaros-Aiolos dimer further includes a fifth Ikaros exon; the fifth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E6, the third exon is E5, the fourth exon is E4, and the fifth exon is E1/2; the peptide is Ikaros Isoform 2.

20 In other preferred embodiments: the Ikaros peptide of the Ikaros-Aiolos dimer further includes a sixth Ikaros exon; the sixth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E6, the third exon is E5, the fourth exon is E4, the fifth exon is E3, and the sixth exon is E1/2; the peptide is Ikaros isoform 1. In preferred
embodiments: the sequence of the Ikaros exon is essentially the same as that of a naturally
occurring Ikaros exon, or a fragment thereof having Ikaros activity; the amino acid
sequence of the Ikaros exon is such that a nucleic acid sequence which encodes it is at least
85%, more preferably at least 90%, yet more preferably at least 95%, and most preferably
25 at least 98 or 99% homologous with a naturally occurring Ikaros exon, or a fragment
thereof having Ikaros activity, e.g., Ikaros having an amino acid sequence represented in
any of SEQ ID NOS:15-21 or SEQ ID NO:22; the amino acid sequence of the Ikaros exon
is such that a nucleic acid sequence which encodes it hybridizes under high or low
stringency to a nucleic acid which encodes a naturally occurring Ikaros exon, or a fragment
30 thereof having Ikaros activity, e.g., an Ikaros exon with the same, or essentially the same,
amino acid sequence as an Ikaros exon represented in any of SEQ ID NOS:15-21 the
amino acid sequence of the Ikaros exon is at least 30, more preferably at least 40, more
preferably at least 50, and most preferably at least 60, 80, 100, or 200 amino acid residues
in length; the encoded Ikaros amino acid sequence is at least 50% more preferably 60%,
35 more preferably 70%, more preferably 80%, more preferably 90%, and most preferably
95% as long as a naturally occurring Ikaros exon, or a fragment thereof having Ikaros
activity; the Ikaros exon is essentially equal in length to a naturally occurring Ikaros exon;
the amino acid sequence of the Ikaros exon is at least 80%, more preferably at least 85%,

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yet more preferably at least 90%, yet more preferably at least 95%, and a most preferably at least 98 or 99% homologous with a naturally occurring Ikaros exon sequence, or a fragment thereof having Ikaros activity, e.g., an Ikaros exon sequence of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21; the Ikaros exon amino acid sequence is the same, or essentially the same, as that of a naturally occurring Ikaros exon, or a fragment of the sequence thereof, e.g., an Ikaros exon described in any of SEQ ID NOS:15-21; and the peptide has Ikaros peptide activity; the peptide has Ikaros antagonist activity.

10 In preferred embodiments: the Ikaros protein of the Ikaros-Aiolos dimer comprises a polypeptide represented by the general formula A-B-C-D-E, wherein A represents Exon 3 or is absent, B represents Exon 4 or is absent, C represents Exon 5 or is absent, D represents Exon 6 or is absent, and E represents Exon 7 or is absent; the polypeptide includes at least two of said exons; the polypeptide includes at least one exon containing a zinc finger domain; the polypeptide includes at least one exon selected from E3, E4 or E5.

15 In preferred embodiments: the exons in the Ikaros peptide of the Ikaros-Aiolos dimer are arranged in the same relative linear order as found in a naturally occurring isoform, e.g., in Ikaros isoform 1, e.g., in a peptide having the exons E3 and E7, E3 is located N-terminal to E7; the linear order of the exons is different from that found in a naturally occurring isoform, e.g., in Ikaros isoform 1, e.g., in a peptide having exons E3, 20 E5, and E7, the direction N-terminal to C-terminal end, is E5, E3, E7; the exons in the peptide differ in one or more of composition (i.e., which exons are present), linear order, or number (i.e., how many exons are present or how many times a given exon is present) from a naturally occurring Ikaros isoform, e.g., from Ikaros isoform 1, 2, 3, 4, or 5; e.g. the Ikaros protein is an isoform generated by *in vitro* exon shuffling.

25 The invention also includes: a cell, e.g., a cultured cell or a stem cell, containing purified Ikaros-protein-encoding-DNA and purified Aiolos-protein-encoding -DNA; a cell capable of expressing an Ikaros and an Aiolos protein; a cell capable of giving rise to a transgenic animal or to a homogeneous population of hemopoietic cells, e.g., lymphoid cells, e.g., T cells; an essentially homogeneous population of cells, each of which includes 30 purified Ikaros-protein-encoding-DNA and purified Aiolos-protein-encoding -DNA; and a method for manufacture of a dimer of the invention including culturing a cell which includes a DNA, preferably a purified DNA, of the invention in a medium to express the peptides.

35 The invention also includes: a preparation of cells, e.g., cultured cells or a stem cells, including a cell containing purified Ikaros-protein-encoding-DNA and a cell encoding purified Aiolos-protein-encoding -DNA.

The invention also includes substantially pure preparation of an antibody, preferably a monoclonal antibody directed against an Ikaros-Aiolos dimer (which preferably does not

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bind to an Ikaros-Ikaros or Aiolos-Aiolos dimer); a therapeutic composition including an Ikaros-Aiolos dimer and a pharmaceutically acceptable carrier; a therapeutic composition which includes a purified DNA of the invention and a pharmaceutically acceptable carrier.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, including administering a therapeutically-effective amount of an Ikaros-Aiolos dimer to the animal.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse including administering to the animal cells selected, e.g., selected in vitro, for the expression of a product of the Ikaros gene and of the Aiolos gene, e.g., hematopoietic stem cells, e.g., cells transformed with Ikaros-peptide-encoding DNA and or Aiolos-peptide-encoding DNA, e.g., hematopoietic stem cells transformed with Ikaros and or Aiolos-peptide-encoding DNA. The Ikaros and Aiolos DNA can be present in the same or in different cells.

In preferred embodiments: the cells are taken from the animal to which they are administered; the cells are taken from an animal which is MHC matched with the animal to which they are administered; the cells are taken from an animal which is syngeneic with the animal to which they are administered; the cells are taken from an animal which is of the same species as is the animal to which they are administered.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, including administering to the animal a nucleic acid encoding an Ikaros peptide and a nucleic acid encoding an Aiolos peptide and expressing the nucleic acids.

In another aspect, the invention features a method of evaluating the effect of a treatment, e.g., a treatment designed to promote or inhibit hematopoiesis, including carrying out the treatment and evaluating the effect of the treatment on the expression of the Ikaros and the Aiolos gene.

In preferred embodiments the treatment is administered: to an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, or a cell, e.g., a cultured stem cell.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Ikaros gene, e.g., a leukemic disorder or other disorder of the immune system, e.g., an immunodeficiency, or a T or B cell related disorder, e.g., a disorder characterized by a shortage of T or B cells,

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including examining the subject for the expression of the Ikaros-Aiolos dimers, non-wild type expression or mis-expression being indicative of risk.

5 In another aspect, the invention features, a method of evaluating an animal or cell model for an immune disorder, e.g., a T cell related disorder, e.g., a disorder characterized by a shortage of T or B cells, including determining if Ikaros-Aiolos dimers in the animal or cell model are expressed at a predetermined level. In preferred embodiments: the predetermined level is lower than the level in a wild type or normal animal; the predetermined level is higher than the level in a wild type or normal animal; or the pattern of isoform expression is altered from wildtype.

10 In another aspect, the invention features a transgenic rodent, e.g., a mouse, having a transgene which includes an Ikaros gene or Ikaros protein encoding DNA and an Aiolos gene or Aiolos protein encoding DNA. In preferred embodiments: the Ikaros and or Aiolos gene or DNA includes a deletion, e.g. a deletion of all or part of one or more exons.

15 In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, immune system disorder, including administering a therapeutically effective amount of an Ikaros-Aiolos dimer to the animal.

20 In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, including administering to the animal cells selected, e.g., selected in vitro, for the production of an Ikaros-Aiolos dimer, e.g., hematopoietic stem cells, e.g., cells transformed with Ikaros and or Aiolos protein-encoding DNA, e.g., hematopoietic stem cells transformed with Ikaros
25 and or Aiolos-protein-encoding DNA.

In preferred embodiments: the cells are taken from the animal to which they are administered; the cells are taken from an animal which is MHC matched with the animal to which they are administered; the cells are taken from an animal which is syngeneic with the animal to which they are administered; the cells are taken from an animal which is of the
30 same species as is the animal to which they are administered.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, including administering to the animal a nucleic acid encoding an Ikaros peptide and a nucleic acid
35 encoding an Aiolos peptide and expressing the nucleic acids.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of an Ikaros-Aiolos dimer, e.g., a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's

disease, including examining the subject for the expression of an Ikaros-Aiolos dimer, non-wild type expression or mis-expression being indicative of risk.

In another aspect, the invention features, a method of inhibiting an interaction, e.g., binding, between a protein, e.g., an Ikaros isoform, Aiolos, an Ikaros-Ikaros dimer, an Aiolos-Aiolos dimer, or a first Ikaros-Aiolos dimer, and a DNA sequence, e.g., a DNA
5 sequence under the control of a δA sequence, an NKFB sequence, a sequence which corresponds to an Ikaros or Aiolos binding site, or a site present in the control region of a lymphocyte restricted gene, e.g., TCR- α , - β , or - δ , CD3 - δ , - ϵ , - γ genes, the SL3 gene, or the HIV LTR gene. The methods includes contacting the DNA sequence with an effective
10 amount of a second Ikaros-Aiolos dimer, e.g., an Ikaros-aiolos dimer described herein.

In another aspect, the invention features, a method of inhibiting an interaction, e.g., binding, between a protein, e.g., an Ikaros isoform, Aiolos, an Ikaros-Ikaros dimer, an Aiolos-Aiolos dimer, or an Ikaros-Aiolos dimer, and a DNA sequence, e.g., a δA sequence, an NKFB sequence, a sequence which corresponds to an Ikaros binding oligonucleotide
15 described herein, or a site present in the control region of a lymphocyte restricted gene, e.g., TCR- α , - β , or - δ , CD3 - δ , - ϵ , - γ genes, the SL3 gene, or the HIV LTR gene. The methods includes contacting the protein with an effective amount of an Ikaros, Aiolos, or Ikaros-Aiolos dimer-binding oligonucleotide.

In another aspect, the invention features, a method of modulating hematopoietic
20 development, e.g., a progression of a cell through a lymphoid lineage, e.g., a lymphocyte maturation and/or function, the method including altering, in a cell or animal, a wild type expression of Ikaros-Aiolos and/or Aiolos-Aiolos dimers.

In preferred embodiments, the expression can be altered by providing Aiolos and/or Ikaros polypeptides.

25 In other preferred embodiments, the method includes supplying to a cell or animal a mutant Aiolos and/or Ikaros polypeptide, e.g., a polypeptide having a dominant negative mutation, e.g., a DNA binding mutation.

In another aspect, the invention features, a method of modulating hematopoietic development, e.g., a progression of a cell through a lymphoid lineage, e.g., a lymphocyte
30 maturation and/or function, the method including altering, in a cell or animal, the ratio of Ikaros-Ikaros dimers to any of Aiolos-Aiolos or Aiolos-Ikaros dimers.

In preferred embodiments, the ratio can be altered by providing Aiolos or Ikaros polypeptides.

35 In other preferred embodiments, the method includes supplying to a cell or animal a mutant Aiolos and/or Ikaros polypeptide, e.g., a polypeptide having a dominant negative mutation, e.g., a DNA binding mutation.

In another aspect, the invention features, a method of modulating hematopoietic development, e.g., a progression of a cell through a lymphoid lineage, e.g., a lymphocyte

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maturation and/or function, the method including altering, in a cell or animal, the ratio of Aiolos-Aiolos dimers to any of Ikaros-Ikaros or Aiolos-Ikaros dimers.

In preferred embodiments, the ratio can be altered by providing Aiolos or Ikaros polypeptides.

5 In other preferred embodiments, the method includes supplying to a cell or animal a mutant Aiolos and/or Ikaros polypeptide, e.g., a polypeptide having a dominant negative mutation, e.g., a DNA binding mutation.

In general, the invention features, a method of providing a proliferation-deregulated cell, or a cell which has non-wild type, e.g., increased, antibody production. The method
10 includes: providing a mammal having a cell which misexpresses Aiolos, e.g., a hematopoietic cell; and isolating a proliferation-deregulated or antibody overexpressing cell from the mammal. The proliferation-deregulated or antibody overexpressing cell can be, e.g., a hematopoietic cell, e.g., a B lymphocyte.

In preferred embodiments: the mammal is a non-human mammal, e.g., a swine, a
15 nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In a preferred embodiment, the method further includes: allowing the Aiolos-misexpressing cell to divide and give rise to a proliferation-deregulated or antibody producing cell, e.g., a lymphocyte; providing a plurality of the proliferation-deregulated
20 cells e.g., lymphocytes or transformed lymphocytes from the mammal.

In preferred embodiments: the proliferation-deregulated or antibody producing cell
20 e.g., a lymphocyte, e.g., a transformed lymphocyte, is isolated from a lymphoma of the mammal.

In preferred embodiments: the mammal is heterozygous at the Aiolos locus; the mammal carries a mutation at the Aiolos gene, e.g., a point mutation in or a deletion for all
25 or part of the Aiolos gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediates DNA binding of the Aiolos protein or for one or more of the two C terminal zinc finger regions which mediate dimerization of the Aiolos protein; the mammal is heterozygous or homozygous for an Aiolos transgene; the mammal carries a mutation in
30 the control region of the Aiolos gene.

In preferred embodiments: the mammal carries a mutation at the Aiolos gene, e.g., a point mutation or a deletion, which, inactivates one or both of transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both
35 of the C terminal Zinc finger domains; the mammal carries deletion for all or part of exon 7.

In preferred embodiments: the proliferation-deregulated or antibody producing cell is a homozygous mutant Aiolos cell e.g., a lymphocyte; the proliferation-deregulated or

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antibody producing lymphocyte is a B lymphocyte; the proliferation-deregulated or antibody producing cell is heterozygous or homozygous for an Aiolos transgene.

In preferred embodiments, the cell is a lymphocyte and is: a cell which secretes one or more anti-inflammatory cytokines; a cell which is antigen or idiotype specific; a cell
5 which produces, or over produces, antibodies, e.g., IgG, IgA, or IgE antibodies.

In a preferred embodiment: the Aiolos-misexpressing cell, e.g., a lymphocyte, is supplied exogenously to the mammal, e.g., to a homozygous wild-type Aiolos mammal or a mammal carrying a mutation at the Aiolos gene, e.g., a point mutation or a deletion for all or part of the Aiolos gene. If exogenously supplied, the cell can be a human or a
10 nonhuman, e.g., a swine, nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, lymphocyte.

In a preferred embodiment the method further comprises isolating one or more cells, e.g., lymphocytes, from the mammal, and allowing the cell or cells to proliferate into a clonal population of cells, e.g., lymphocytes.

In preferred embodiments: the mammal is immunized with an antigen; the cell is exogenously supplied and one or both of the mammal or the mammal which donates the cell are immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen; an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

In preferred embodiments the method further includes providing a lymphocyte e.g.,
20 a B lymphocyte, or a substantially homogenous population of lymphocytes, e.g., B lymphocytes, which produce an antibody molecule, e.g. an IgG, IgA, or IgE molecule, which recognizes a selected antigen.

In another aspect, the invention features, a method of providing a proliferation-deregulated cell, or a cell which has non-wild type, e.g., increased, antibody production.
25 The method includes: causing a subject cell to misexpress the Aiolos gene, e.g., by inducing an Aiolos mutation, thereby providing a proliferation-deregulated or antibody overexpressing cell. The proliferation-deregulated or antibody overexpressing cell can be, e.g., a hematopoietic cell, e.g., a B lymphocyte.

In preferred embodiments: the subject cell is from a non-human mammal, e.g., a
30 swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In a preferred embodiment, the method further includes: allowing the Aiolos-misexpressing cell to divide and give rise to a proliferation-deregulated or antibody producing cell, e.g., a lymphocyte; providing a plurality of the proliferation-deregulated cells e.g., lymphocytes or transformed lymphocytes from the mammal.

In preferred embodiments: the proliferation-deregulated or antibody producing cell
35 e.g., a lymphocyte, e.g., a transformed lymphocyte, is isolated from cell or tissue culture.

In preferred embodiments: the cell is heterozygous at the Aiolos locus; the cell carries a mutation at the Aiolos gene, e.g., a point mutation in or a deletion for all or part of

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the Aiolos gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediates DNA binding of the Aiolos protein or for one or more of the two C terminal zinc finger regions which mediate dimerization of the Aiolos protein; the mammal is

5 heterozygous or homozygous for an Aiolos transgene; the cell carries a mutation in the control region of the Aiolos gene.

In preferred embodiments: the cell carries a mutation at the Aiolos gene, e.g., a point mutation or a deletion, which, inactivates one or both of transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both

10 of the C terminal Zinc finger domains; the mammal carries deletion for all or part of exon 7.

In preferred embodiments: the proliferation-deregulated or antibody producing cell is a homozygous mutant Aiolos cell e.g., a lymphocyte; the proliferation-deregulated or antibody producing lymphocyte is a B lymphocyte; the proliferation-deregulated or

15 antibody producing cell is heterozygous or homozygous for an Aiolos transgene.

In preferred embodiments, the cell is a lymphocyte and is: a cell which secretes one or more anti-inflammatory cytokines; a cell which is antigen or idotype specific; a cell which produces, or over produces, antibodies, e.g., IgG, IgA, or IgE antibodies.

In a preferred embodiment the method further comprises allowing the subject cell,

20 to proliferate into a clonal population of cells, e.g., lymphocytes.

In preferred embodiments: the mammal which supplies the subject cell is immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen; an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

In preferred embodiments the method further includes providing a lymphocyte e.g.,

25 a B lymphocyte, or a substantially homogenous population of lymphocytes, e.g., B lymphocytes, which produce an antibody molecule, e.g. an IgG, IgA, or IgE molecule, which recognizes a selected antigen.

In another aspect, the invention features, a cell, e.g., a hematopoietic cell, e.g., a B lymphocyte, or, a clonal population or substantially purified preparation of such cells,

30 preferably produced by a method of the invention described herein. Preferably, the cells misexpress Aiolos.

In another aspect, the invention features, a cell which produces or over produces an antibody, e.g., an IgA, IgG, or IgE antibody. The cell can be, e.g., a hematopoietic cell, e.g., a B lymphocyte, or a population, or substantially purified preparation, of such cells ,

35 preferably produced by a method of the invention described herein. Preferably the cells misexpress Aiolos.

In another aspect, the invention features, a proliferation-deregulated cell. The cell can be, e.g., a hematopoietic cell, e.g., a B lymphocyte, or a population, or substantially

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purified preparation, of such cells, preferably produced by a method of the invention described herein. Preferably the cells misexpress Aiolos.

In another aspect, the invention features, a lymphocyte, e.g., a B lymphocyte, or, a substantially homogenous population or substantially purified preparation of lymphocytes, preferably produced by a method of the invention described herein, which lymphocytes or
5 population recognize a selected antigen. Preferably, the lymphocytes misexpress Aiolos.

In another aspect, the invention features, a method of culturing an Aiolos-misexpressing cell having at least one mutant allele at the Aiolos locus. The cell can be, e.g., a hematopoietic cell, e.g., a B lymphocyte. The method includes: introducing the cell
10 into a mammal, wherein, preferably, the mammal is other than the one from which the cell has been isolated originally; and culturing the cell.

In a preferred embodiment, the method further includes: allowing the cell to proliferate in the mammal.

In preferred embodiments: the mammal is a non-human mammal, e.g., a swine, a
15 nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In a preferred embodiment, the method further includes: allowing the Aiolos-misexpressing cell to divide and give rise to a proliferation-deregulated cell, e.g., a transformed lymphocyte; providing a plurality of the proliferation-deregulated cells e.g., lymphocytes or transformed lymphocytes from the mammal.

In preferred embodiments: the mammal, the cell or both, are heterozygous at the
20 Aiolos locus; the mammal, the cell or both, carry a mutation at the Aiolos gene, e.g., a point mutation in or a deletion for all or part of the Aiolos gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediates DNA binding of the Aiolos protein or
25 for one or more of the two C terminal zinc finger regions which mediate dimerization of the Aiolos protein; the mammal is heterozygous or homozygous for an Aiolos transgene; the mammal, the cell or both, carry a mutation in the control region of the Aiolos gene.

In preferred embodiments: the mammal, the cell or both, carry a mutation at the Aiolos gene, e.g., a point mutation or a deletion, which, inactivates one or both of
30 transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains; the mammal, the cell or both, carry a deletion for all or part of exon 7.

In preferred embodiments: the Aiolos-misexpressing cell is a homozygous mutant Aiolos cell e.g., a lymphocyte; the Aiolos-misexpressing cell is a B lymphocyte; the
35 Aiolos-misexpressing cell is heterozygous or homozygous for an Aiolos transgene.

In preferred embodiments, the Aiolos-misexpressing cell is a lymphocyte and is: a cell which secretes one or more anti-inflammatory cytokines; a cell which is antigen or

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idiotype specific; a cell which produces, or over produces, antibodies, e.g., IgG, IgA, or IgE antibodies.

5 In preferred embodiments: the mammal is immunized with an antigen; the cell is exogenously supplied and one or both of the mammal or the mammal which donates the cell are immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen; an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

10 In a preferred embodiment: the Aiolos-misexpressing cell, e.g., a lymphocyte, is supplied exogenously to the mammal, e.g., to a homozygous wild-type Aiolos mammal or a mammal carrying a mutation at the Aiolos gene, e.g., a point mutation or a deletion for all or part of the Aiolos gene. If exogenously supplied, the cell can be a human or a nonhuman, e.g., a swine, nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, lymphocyte.

Aiolos wild type cells can be cultured in Aiolos misexpressing mammals.

15 In another aspect, the invention features, a method of modulating the activity of, or promoting the interaction of an Aiolos misexpressing cell with, a target tissue or cell. The method includes: supplying the target; and exposing the target to a Aiolos misexpressing cell, e.g., a hematopoietic cell, e.g., a B lymphocyte, preferably having at least one mutant allele at the Aiolos locus, preferably provided that: the target is not Aiolos-misexpressing; the target and the cell differ in genotype at a locus other than the Aiolos locus; the target
20 and the cell are from different animals; the target and the cell are from different species; the target activity is modulated in a recipient mammal and either the target or the cell is from a donor mammal other than the recipient mammal; or the target is exposed to the cell in an *in vitro* system.

25 In a preferred embodiment: the donor of the Aiolos-misexpressing cell is heterozygous or homozygous for an Aiolos transgene; the donor of the Aiolos-misexpressing cell is heterozygous at the Aiolos locus; the donor of the Aiolos-misexpressing cell carries a point mutation in or a deletion for all or part of the Aiolos gene, e.g., mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediate Aiolos
30 binding to DNA or in one or both of the C-terminal zinc finger regions which mediate Aiolos dimerization; the donor of the Aiolos-misexpressing cell is human or a non-human mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a rat or a mouse. In preferred embodiments, e.g., in the case of the human donor, the manipulation that gives rise to Aiolos deregulation, e.g., an Aiolos lesion, can be made *in vitro*.

35 In preferred embodiments: the mammal which provides the Aiolos misexpressing cell carries a mutation at the Aiolos gene, e.g., a point mutation or a deletion, which, inactivates one or both of transcriptional activation or dimerization, which decreases the

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half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains; the mammal carries deletion for all or part of exon 7.

In another preferred embodiment: the cell is heterozygous or homozygous for an Aiolos transgene; the cell is a heterozygous Aiolos cell; the cell is a homozygous mutant Aiolos cell; the lymphocyte is a B lymphocyte.

In preferred embodiments, the cell is a lymphocyte and is: a B cell; a cell which secretes one or more anti-inflammatory cytokines; a T cell which is antigen or idiotype specific.

In a preferred embodiment: the method is performed in an *in vitro* system; the method is performed *in vivo*, e.g., in a mammal, e.g., a rodent, e.g., a mouse or a rat, or a primate, e.g., a non-human primate or a human. If the method is performed *in vitro*, the donor of the target cell or tissue and the lymphocyte can be same or different. If the method is performed *in vivo*, there is a recipient animal and one or more donors.

In preferred embodiments: the method is performed *in vivo* and one or more of the recipient, the donor of the target cell or tissue, the donor of the cell, is immunized with an antigen; the method is performed *in vitro* and one or more of the donor of the target cell or tissue, the donor of the cell is immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

In a preferred embodiment: the target is selected from a group consisting of T or B lymphocytes, macrophages, inflammatory leukocytes, e.g., neutrophils or eosinophils, mononuclear phagocytes, NK cells or T lymphocytes; the target is an antigen presenting cell, e.g., a professional antigen presenting cell or a nonprofessional antigen presenting cell; the target is spleen tissue, bone marrow tissue, lymph node tissue or thymic tissue, or the target is a syngeneic, allogeneic, or xenogeneic tissue.

In another preferred embodiment, the target is from a mammal, e.g., a human; the mammal is a non-human mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In preferred embodiments, the activity of the target which is modulated is: the production of a cytokine; the proliferation or activation of a cell of the immune system; the production of an antibody; the lysis of an antigen presenting cell or the activation of a cytolytic T lymphocyte; the effect of target on resistance to infection; the effect of target on life span; the effect of target on body weight; the effect of target on the presence, function, or morphology of tissues or organs of the immune system; the effect of target on the ability of a component of the immune system to respond to a stimulus (e.g., a diffusable substance, e.g., cytokines, other cells of the immune system, or antigens); the effect of target on the ability to exhibit immunological tolerance to an alloantigen or a xenoantigen.

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In preferred embodiments the interaction is the binding of an antibody produced by the Aiolos misexpressing cell with the target.

In preferred embodiments: the target and the cell differ in genotype at a locus other than the Aiolos locus; the target and the cell are from different animals; the target is not
5 Aiolos-misexpressing.

In another aspect, the invention features, a method of reconstituting an immune system. The method includes: supplying a recipient mammal, and introducing, preferably exogenously, into the recipient mammal, an immune system component from a donor mammal, which is Aiolos misexpressing, e.g., which carries at least one mutant allele at the
10 Aiolos locus. The recipient mammal, can be, e.g., a human or a nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse. The donor mammal can be, e.g., a human or a nonhuman mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a rat or a mouse. If the donor mammal is human, the manipulation that gives rise to Aiolos misexpression e.g., an the introduction of an Aiolos lesion, can be
15 made *in vitro*. The donor mammal and the recipient mammal can be different individuals or the same individual.

In preferred embodiments, the component is or includes an Aiolos misexpressing cell, e.g., a hematopoietic cell, e.g., a pluripotent stem cell, or a descendent of a stem cell, e.g., a lymphocyte.

20 In preferred embodiments, the component is from a donor mammal, e.g., a human or a nonhuman mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In a preferred embodiment, the method further includes: prior to introduction of a component into the subject, treating the lymphocyte to inhibit proliferation, e.g., by irradiating the component.

25 In a preferred embodiment, the donor mammal carries a mutation at the Aiolos gene, e.g., a deletion of all or part of the Aiolos gene.

In another preferred embodiment: the immune system component is any of a T cell, a T cell progenitor, a totipotent hematopoietic stem cell, a pluripotent hematopoietic stem cell, a B cell, a B cell progenitor, a natural killer cell, a natural killer cell progenitor, bone
30 marrow tissue, spleen tissue, or thymic tissue.

In a preferred embodiment: the immune system component is from the same species as the recipient mammal; the immune system component is from species different from the species of the recipient mammal.

In preferred embodiments: the recipient mammal is a wild-type animal; an animal
35 model for a human disease, e.g., a NOD mouse; the animal is immunocompromised by irradiation, chemotherapy, or genetic defect, e.g., the animal is a SCID mouse or a nude mouse; the recipient is deficient in an immune function, e.g., the recipient has been

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thymectomized, depleted of an immune system component, e.g., of cells or antibodies; the recipient has been administered chemotherapy or irradiation.

In preferred embodiments: the immune system component is heterozygous at the Aiolos locus; the immune system component carries a mutation at the Aiolos gene, e.g., a point mutation in or a deletion for all or part of the Aiolos gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediate DNA binding of the Aiolos protein or for one or more of the two C terminal zinc finger regions which mediate dimerization of the Aiolos protein; the immune system component is heterozygous or homozygous for an Aiolos transgene; the immune system component carries a mutation in the control region of the Aiolos gene.

In preferred embodiments: the immune system component carries a mutation at the Aiolos gene, e.g., a point mutation or a deletion, which inactivates one or both of transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains; the immune system component carries deletion for all or part of exon 7.

In preferred embodiments: the method is performed *in vivo*, and the recipient mammal or the donor mammal or both are immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

In a preferred embodiment, the method further includes: determining a value for a parameter related to immune system function. The parameter related to the immune system function can be any of: the production of a cytokine; the proliferation or activation of a cell of the immune system; the production of an antibody; the lysis of an antigen presenting cell or the activation of a cytolytic T lymphocyte; resistance to infection; life span; body weight; the presence, function, or morphology of tissues or organs of the immune system; the ability of a component of the immune system to respond to a stimulus (e.g., a diffusable substance, e.g., cytokines, other cells of the immune system, or antigens); the ability to present an antigen; the ability to exhibit immunological tolerance to an alloantigen or a xenoantigen.

In another aspect, the invention features, a method of evaluating the interaction of an Aiolos misexpressing cell, e.g., a hematopoietic cell, a B lymphocyte, with an immune system component. The method includes: supplying an animal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse; introducing the cell and the immune component into the animal; and evaluating the interaction between the Aiolos misexpressing cell and the immune system component.

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In a preferred embodiment, the method further includes: prior to introduction of a cell into the subject, treating the lymphocyte to inhibit proliferation, e.g., by irradiating the cell.

5 In a preferred embodiment: the immune system component is any of a T cell, a T cell progenitor, a totipotent hematopoietic stem cell, a pluripotent hematopoietic stem cell, a B cell, a B cell progenitor, a natural killer cell, a natural killer cell progenitor, bone marrow tissue, spleen tissue, or thymic tissue; the immune system component is from the same species as the animal; the immune system component is from species different from the species of the animal; the immune system component is from the same species as the lymphocyte; the immune system component is from species different from the species from
10 which the lymphocyte is obtained.

In another preferred embodiment: the cell is from the same species as the animal; the cell is from a species which is different from the species of the animal.

In another preferred embodiment: the recipient mammal is a wild-type animal; an
15 animal model for a human disease, e.g., a NOD mouse; the animal is immunocompromised by irradiation, chemotherapy, or genetic defect, e.g., the animal is a SCID mouse or a nude mouse; the recipient is deficient in an immune function, e.g., the recipient has been thymectomized, depleted of an immune system component, e.g., of cells or antibodies; the recipient has been administered chemotherapy or irradiation.

20 In a preferred embodiment: the cell is heterozygous or homozygous for an Aiolos transgene.

In preferred embodiments evaluating can include evaluating any of: the production of a cytokine; the proliferation or activation of a cell of the immune system; the production of an antibody; the lysis of an antigen presenting cell or the activation of a cytolytic T
25 lymphocyte; resistance to infection; life span; body weight; the presence, function, or morphology of tissues or organs of the immune system; the ability of a component of the immune system to respond to a stimulus (e.g., a diffusible substance, e.g., cytokines, other cells of the immune system, or antigens); the ability to present an antigen; the ability to exhibit immunological tolerance to an alloantigen or a xenoantigen.

30 In preferred embodiments: the method is performed *in vivo*, and one or more of the animal, the donor of the Aiolos misexpressing cell, the donor of the immune system component, is immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

35 In another aspect, the invention features, a mammal, e.g., a nonhuman mammal, e.g., e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, having an exogenously introduced immune system component, the component being from a human or nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a

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monkey, a goat, or a rodent, e.g., a rat or a mouse, or cell culture which is Aiolos misexpressing or which carries at least one mutant allele at the Aiolos locus. In preferred embodiments, e.g., if the immune system component is from a wild-type animal, e.g., a human, the manipulation that gives rise to Aiolos deregulation, e.g., an Aiolos lesion, can be made *in vitro*.

In preferred embodiments, the component is from a human or nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, which is Aiolos misexpressing.

In preferred embodiments: the component is from a mammal which is Aiolos misexpressing; the component is from a mammal which is heterozygous at the Aiolos locus; the component is from a mammal which carries a mutation at the Aiolos gene, e.g., a point mutation in or a deletion for all or part of the Aiolos gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediates DNA binding of the Aiolos protein or for one or more of the two C terminal zinc finger regions which mediate dimerization of the Aiolos protein; the component is from a mammal which is heterozygous or homozygous for an Aiolos transgene; the component is from a mammal which carries a mutation in the control region of the Aiolos gene.

In preferred embodiments: the component is from a mammal which carries a mutation at the Aiolos gene, e.g., a point mutation or a deletion, which, inactivates one or both of transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains; the component is from a mammal which carries deletion for all or part of exon 7.

In preferred embodiments, the immune system component is: a helper T cell; cytolytic T cell; a suppressor T cell; a T cell which secretes one or more anti-inflammatory cytokines, e.g., IL-4, IL-10, or IL-13; a T cell which is antigen or idiotypic specific; a suppressor T cell which is anti-idiotypic for an auto antibody or for an antibody which recognizes an allograft or xenograft tissue; the lymphocyte is an antigen-nonspecific T cell.

In another preferred embodiment: the immune system component is any of a T cell progenitor, a totipotent hematopoietic stem cell, a pluripotent hematopoietic stem cell, a B cell, a B cell progenitor, a natural killer cell, a natural killer cell progenitor, bone marrow tissue, spleen tissue, or thymic tissue; the immune system component is from the same species as the animal; the immune system component is from species different from the species of the animal.

In preferred embodiments: the mammal or the donor animal which produces the immune system component or both are immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

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In another aspect, the invention features, a reaction mixture, preferably an *in vitro* reaction mixture, including an immune system component, the component including cells which misexpress Aiolos or being from an animal or cell culture which misexpresses Aiolos or which carries at least one mutant allele at the Aiolos locus, and a target tissue or cell, wherein preferably, the immune system component and the target differ in genotype at a locus other than the Aiolos or Ikaros locus; the component and the target are from different species, or the component and the target are from different animals.

In preferred embodiments, the component is from an animal or cell culture which misexpresses Aiolos.

10 In preferred embodiments: the immune system component is a lymphocyte heterozygous or homozygous for an Aiolos transgene, e.g., a transgene having a point mutation or a deletion, which, inactivates one or both of transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains; the immune system component is a lymphocyte
15 heterozygous or homozygous for a C terminal deletion.

In preferred embodiments, the immune system component is: a B cell.

In another preferred embodiment: the immune system component is any of a T cell progenitor, a totipotent hematopoietic stem cell, a pluripotent hematopoietic stem cell, a B cell, a B cell progenitor, a natural killer cell, a natural killer cell progenitor, bone marrow
20 tissue, spleen tissue, or thymic tissue; the immune system component is from the same species as the target cell; the immune system component is from species different from the species of the target cell.

In a preferred embodiment: the target is selected from a group consisting of T or B lymphocytes, macrophages, inflammatory leukocytes, e.g., neutrophils or eosinophils, mononuclear phagocytes, NK cells or T lymphocytes; the target is an antigen presenting cell, e.g., a professional antigen presenting cell or a nonprofessional antigen presenting cell; the target is spleen tissue, lymph node tissue, bone marrow tissue or thymic tissue, or is syngeneic, allogeneic, xenogeneic, or congenic tissue.

25 In preferred embodiments: the donor of the immune system component or the donor of the target or both are immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

30 In preferred embodiments the donor of the components is: a human or nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or mouse. In preferred embodiments, e.g., in the case of a wild-type donor, e.g., a human, the manipulation that gives rise to Aiolos deregulation, e.g., an Aiolos lesion, can be introduced *in vitro*.
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In preferred embodiments the donor of the target is: a human or nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or mouse.

In preferred embodiments the reaction mixture includes an exogenously add
5 cytokine or antigen, e.g., a protein antigen.

In another aspect, the invention features, a method of promoting or inhibiting the proliferation of a cell, or of modulating the entry of a cell into the cell cycle. The method includes: administering to the cell a compound which inhibits the formation Aiolos-Aiolos or Aiolos-Ikaros dimers. The method can be performed in vivo or in vitro. The cell can be,
10 e.g., a hematopoietic cell, e.g., a B lymphocyte

In preferred embodiments, the compound is: a competitive or noncompetitive inhibitor of the association of Aiolos or Ikaros subunits, e.g., a mutant Aiolos peptide, e.g., a mutant Aiolos peptide which has a mutation which inhibits the ability of the Aiolos protein to bind DNA but which does not inhibit the ability of the protein to form a dimer,
15 e.g., a mutation in one or more of the four N terminal Zinc fingers binding regions. Aiolos mutants which have mutations which inhibit dimerization, e.g., mutations in one of more of the two C terminal zinc finger regions can also be used.

In preferred embodiments the compound is: a protein or peptide; a peptomimetic, a small molecule; a nucleic acid which encodes an inhibitor.

20 Methods for increasing cell division can be combined with procedures where it is desirable to increase cell division, e.g., the treatment, e.g., by chemotherapy or radiotherapy, of tumors or other cell-proliferative disorders.

Proliferation can be inhibited by administering wildtype Aiolos.

In another aspect, the invention features a cell, or purified preparation of cells,
25 which include an Aiolos transgene, or which otherwise misexpress an Aiolos gene. The cell preparation can consist of human or non human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include an Aiolos transgene, e.g., a heterologous form of an Aiolos gene, e.g., a gene derived from humans (in the case of a non-human cell). The Aiolos transgene can be misexpressed, e.g.,
30 overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous Aiolos gene, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed Aiolos alleles or for use in drug screening.

Cells, e.g., stem cells, treated by the method of the invention can be introduced into
35 mammals, e.g., humans, non-human primates, or other mammals, e.g., rodents. In preferred embodiments the treatment is performed *ex vivo* and: the cell is autologous, e.g., it is returned to the same individual from which it was derived; the cell is allogeneic, i.e., it

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is from the same species as the mammal to which it is administered; the cell is xenogeneic, i.e., it is from a different species from the mammal to which it is administered.

An Aiolos-deregulated cell is a cell which has a mutant or misexpressed Aiolos gene, e.g., an inactivated Aiolos gene.

5 A hematopoietic cell, can be, e.g., stem cell, e.g., a totipotent or a pluripotent stem cell, or a descendent of a stem cell, e.g., a lymphocyte, e.g. a B lymphocyte or a T lymphocyte.

A proliferation-deregulated cell, as used herein, refers to a cell with other than wild

10 An Aiolos misexpressing animal, as used herein, is an animal in which one or more, and preferably substantially all, of the cells misexpress Aiolos.

A mutation at the Aiolos locus, as used herein, includes any mutation which alters the expression, structure, or activity of the Aiolos gene or its gene product. These include point mutations in and in particular deletions of all or part of the Aiolos coding region or its control region.

15 An exogenously supplied cell, tissue, or cell product, e.g., a cytokine, as used herein, is a cell, tissue, or a cell product which is derived from an animal other than the one to which is supplied or administered. It can be from the same species or from different species than the animal to which it is supplied.

20 A clonal population of lymphocytes, as used herein, is a population of two or more lymphocytes which have one or more of the following properties: they share a common stem cell ancestor; they share a common pre-thymocyte or pre b cell ancestor; they share a common thymocyte ancestor; they share the same T cell receptor genomic rearrangement; they share a common CD4+CD8+ ancestor; they share a common CD4+ ancestor; they share a common CD8+ ancestor; they share a common CD4-CD8- ancestor; they recognize
25 the same antigen.

A substantially homogenous population of two or more cells e.g., lymphocytes, as used herein, means a population of cells in which at least 50% of the cells, more preferably at least 70% of the cells, more preferably at least 80% of the cells, most preferably at least 90%, 95% or 99% of the subject cell type, e.g., lymphocytes. With respect to the Aiolos
30 locus however, the cells can be all (+/-), all (-/-), or a mixture of (+/-) and (-/-) cells.

Culturing, as used herein, means contacting a cell or tissue with an environment which will support viability of the cell or tissue and which preferably supports proliferation of the cell or tissue.

35 A substantially purified preparation of cells, e.g., lymphocytes, as used herein, means a preparation of cells in which at least 50% of the cells, more preferably at least 70% of the cells, more preferably at least 80% of the cells, most preferably at least 90%, 95% or 99% of the cells of the subject cell, e.g., are lymphocytes. With respect to the Aiolos locus however, the cells can be all (+/-), all (-/-), or a mixture of (+/-) and (-/-) cells.

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Immunocompromised, as used herein, refers to a mammal in which at least one aspect of the immune system functions below the levels observed in a wild-type mammal. The mammal can be immunocompromised by a chemical treatment, by irradiation, or by a genetic lesion resulting in, e.g., a nude, a beige, a nude-beige, or an Ikaros - phenotype.

- 5 The mammal can also be immunocompromised by an acquired disorder, e.g., by a virus, e.g., HIV.

As used herein, an Aiolos transgene, is a transgene which includes all or part of an Aiolos coding sequence or regulatory sequence. The term also includes DNA sequences which when integrated into the genome disrupt or otherwise mutagenize the Aiolos locus.

10 Aiolos transgenes sequences which when integrated result in a deletion of all or part of the Aiolos gene. Included are transgenes: which upon insertion result in the misexpression of an endogenous Aiolos gene; which upon insertion result in an additional copy of an Aiolos gene in the cell; which upon insertion place a non-Aiolos gene under the control of an Aiolos regulatory region. Also included are transgenes: which include a copy of the Aiolos

15 gene having a mutation, e.g., a deletion or other mutation which results in misexpression of the transgene (as compared with wild type); which include a functional copy of an Aiolos gene (i.e., a sequence having at least 5% of a wild type activity, e.g., the ability to support the development of T, B, or NK cells); which include a functional (i.e., having at least 5% of a wild type activity, e.g., at least 5% of a wild type level of transcription) or

20 nonfunctional (i.e., having less than 5% of a wild type activity, e.g., less than a 5% of a wild type level of transcription) Aiolos regulatory region which can (optionally) be operably linked to a nucleic acid sequence which encodes a wild type or mutant Aiolos gene product or, a gene product other than an Aiolos gene product, e.g., a reporter gene, a toxin gene, or a gene which is to be expressed in a tissue or at a developmental stage at

25 which Aiolos is expressed. Preferably, the transgene includes at least 10, 20, 30, 40, 50, 100, 200, 500, 1,000, or 2,000 base pairs which have at least 50, 60, 70, 80, 90, 95, or 99 % homology with a naturally occurring Aiolos sequence. Preferably, the transgene includes a deletion of all or some of exons 3 and 4, or a deletion for some or all of exon 7 of the Aiolos gene.

- 30 A "heterologous promoter", as used herein is a promoter which is not naturally associated with the Aiolos gene.

A "purified preparation" or a "substantially pure preparation" of an Aiolos polypeptide, or a fragment or analog thereof (or an Aiolos-Aiolos or Aiolos-Ikaros dimer), as used herein, means an Aiolos polypeptide, or a fragment or analog thereof (or an Aiolos-

35 Aiolos or Aiolos-Ikaros dimer), which is free of one or more other proteins lipids, and nucleic acids with which the Aiolos polypeptide (or an Aiolos-Aiolos or Aiolos-Ikaros dimer) naturally occurs. Preferably, the polypeptide, or a fragment or analog thereof (or an Aiolos-Aiolos or Aiolos-Ikaros dimer), is also separated from substances which are used to

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purify it, e.g., antibodies or gel matrix, such as polyacrylamide. Preferably, the polypeptide, or a fragment or analog thereof (or an Aiolos-Aiolos or Aiolos-Ikaros dimer), constitutes at least 10, 20, 50 70, 80 or 95% dry weight of the purified preparation.

Preferably, the preparation contains: sufficient polypeptide to allow protein sequencing; at least 1, 10, or 100 µg of the polypeptide; at least 1, 10, or 100 mg of the polypeptide.

A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an *in vitro* preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

A "treatment", as used herein, includes any therapeutic treatment, e.g., the administration of a therapeutic agent or substance, e.g., a drug.

A "substantially pure nucleic acid", e.g., a substantially pure DNA encoding an Aiolos polypeptide, is a nucleic acid which is one or both of: not immediately contiguous with one or both of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the nucleic acid is derived; or which is substantially free of a nucleic acid sequence with which it occurs in the organism from which the nucleic acid is derived. The term includes, for example, a recombinant DNA which is incorporated into a vector, e.g., into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. Substantially pure DNA also includes a recombinant DNA which is part of a hybrid gene encoding additional Aiolos sequences.

"Homologous", as used herein, refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared x 100. For example, if 6 of 10, of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

The terms "peptides", "proteins", and "polypeptides" are used interchangeably herein.

As used herein, the term "transgene" means a nucleic acid sequence (encoding, e.g., one or more Aiolos polypeptides or Aiolos-Ikaros dimers), which is partly or entirely

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heterologous, i.e., foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of the selected nucleic acid, all operably linked to the selected nucleic acid, and may include an enhancer sequence.

10 As used herein, the term "transgenic cell" refers to a cell containing a transgene.

As used herein, a "transgenic animal" is any animal in which one or more, and preferably essentially all, of the cells of the animal includes a transgene. The transgene can be introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA.

As used herein, the term "tissue-specific promoter" means a DNA sequence that serves as a promoter, i.e., regulates expression of a selected DNA sequence, such as the Aiolos and/or Ikaros gene, operably linked to the promoter, and which effects expression of the selected DNA sequence in specific cells of a tissue, such as lymphocytes. The term also covers so-called "leaky" promoters, which regulate expression of a selected DNA primarily in one tissue, but cause expression in other tissues as well.

A polypeptide has Aiolos biological activity if it has one or more of the following properties: (1) the ability to react with an antibody, or antibody fragment, specific for (a) a wild type Aiolos polypeptide, (b) a naturally-occurring mutant Aiolos polypeptide, or (c) a fragment of either (a) or (b); (2) the ability to form Aiolos dimers and/or Aiolos/Ikaros dimers; (3) the ability to modulate lymphocyte differentiation; (4) the ability to stimulate transcription from a sequence, e.g., a sequence described herein; or (5) the ability to act as an antagonist or agonist of the activities recited in (1), (2), (3) or (4).

30 "Misexpression", as used herein, refers to a non-wild type pattern of Aiolos gene expression. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing, size, amino acid sequence, post-translational modification, stability, or biological activity of the expressed Aiolos and/or Ikaros polypeptide; a pattern of expression that

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differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the Aiolos and/or Ikaros gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus; a ratio of Ikaros-Ikaros dimer to Aiolos-Aiolos dimer which differs from wild type; a ratio of Aiolos to Aiolos-Aiolos dimer, Ikaros-Ikaros dimer, or Ikaros-Aiolos dimer that differs from wild type; a ratio of Ikaros-Aiolos dimer to Aiolos, Ikaros, Aiolos-Aiolos dimer, or Ikaros-Ikaros dimer that differs from wild type.

As described herein, one aspect of the invention features a pure (or recombinant) nucleic acid which includes a nucleotide sequence encoding an Aiolos, and/or equivalents of such nucleic acids. The term "nucleic acid", as used herein, can include fragments and equivalents. The term "equivalent" refers to nucleotide sequences encoding functionally equivalent polypeptides or functionally equivalent polypeptides which, for example, retain the ability to react with an antibody specific for an Aiolos polypeptide. Equivalent nucleotide sequences will include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants, and will, therefore, include sequences that differ from the nucleotide sequence of Aiolos shown in SEQ ID NO:1 or SEQ ID NO:7 due to the degeneracy of the genetic code.

An Aiolos-responsive control element, as used herein is a region of DNA which, when present upstream or downstream from a gene, results in regulation, e.g., increased transcription of the gene in the presence of an Aiolos protein.

A peptide has Ikaros activity if it has one or more of the following properties: the ability to stimulate transcription of a DNA sequence under the control any of a δA element, an NF κ B element, or one of the Ikaros binding oligonucleotide consensus sequences disclosed herein; the ability to bind to any of a δA element, an NF κ B element, or one of the Ikaros binding oligonucleotide consensus sequences disclosed herein; or the ability to competitively inhibit the binding of a naturally occurring Ikaros isoform to any of a δA element, an NF κ B element, or one of the Ikaros binding oligonucleotide consensus sequences disclosed herein. An Ikaros peptide is a peptide with Ikaros activity.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, *Molecular Cloning A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); *DNA Cloning*, Volumes I and II (D. N. Glover ed., 1985); *Oligonucleotide Synthesis* (M. J. Gait ed., 1984); Mullis et al. U.S. Patent No. 4,683,195; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B.

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Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu et al. eds.), *Immunochemical Methods In Cell And*
 5 *Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

The Aiolos genes and polypeptides of the present invention are useful for studying,
 10 diagnosing and/or treating diseases associated with unwanted cell proliferation, e.g., leukemias or lymphomas. The gene (or fragment thereof) can be used to prepare antisense constructs capable of inhibiting expression of a mutant or wild type Aiolos gene encoding a polypeptide having an undesirable function. Alternatively, an Aiolos polypeptide can be
 15 used to raise antibodies capable of detecting proteins or protein levels associated with abnormal cell proliferation or lymphocyte differentiation, e.g., T cell maturation. Furthermore, Aiolos peptides, antibodies or nucleic acids, can be used to identify the stage of lymphocyte differentiation, e.g., the stage of T cell differentiation.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

20 Brief Description of the Figures

Fig. 1 is a diagram depicting mouse Aiolos cDNA. 1A: is a mouse Aiolos cDNA nucleotide sequence. 1B: is a corresponding amino acid sequence 507 amino acids in length.

Fig. 2 is a diagram depicting homology at the amino acid level between the mouse and chicken Aiolos sequence and the mouse and chicken Ikaros exon 7 sequence.

25 Fig. 3 is a diagram depicting the homology between mouse Aiolos amino acid sequence and mouse Ikaros amino acid sequence.

Fig. 4 is a diagram depicting Aiolos exons. Based on homology to Ikaros, the exons encoding different segments of the Aiolos gene are deduced. The exon boundaries of exons 5/6 and 6/7 have been confirmed from genomic sequence (6/7) or from differential splice
 30 products (5/6). Three classes of cDNA were recovered. The first contains exons 3 through 7. A second class splices exon 5 directly to exon 7, skipping exon 6. The third contains exon 7 and contiguous genomic sequence extending upstream of this exon.

Fig. 5A: is a human Aiolos cDNA nucleotide sequence. Consensus sequence of human Aiolos cDNA from RTPCR using mouse AioF primer (ex3) in forward direction
 35 and human hAio2 primer (ex6) in reverse direction. This sequence does not include the AioF primer sequence but does include the hAio2 sequence. AioF = atg aaa gtg aaa gat gaa tac agc only human sequence is shown here. EcoRI sites flank directly 5' and 3'. The cDNA sequence in figure 5A is SEQ ID NO: 7. 5B: shows a corresponding human amino

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acid sequence 209 amino acids in length. 5B also shows the corresponding mouse sequence and shows regions of shared sequence. The human protein sequence in 5B is SEQ ID NO: 8

5 Fig. 6 is a diagram depicting comparison of the amino acid sequence of Aiolos (top sequence) and Ikaros (bottom sequence) proteins. The boxed methionines represent the three translation initiation codons. The boxed cysteines and histidines represent the paired cysteines and histidines of the zinc finger motifs. The conserved activation domain (amino acids 290-344 of Aiolos protein) is shaded. Identical residues are indicated by bars and conservative residues are indicated by dots.

10 Fig. 7 is a bar graph depicting the effect of different isoforms on the transcriptional activation of Ikaros.

Fig. 8 is a schematic diagram depicting a model for the role of Aiolos and Ikaros in the progression of the lymphoid lineage.

Detailed Description of the Invention

Overview

15 The development of lymphocytes is dependent on the activity of the zinc finger transcription factor Ikaros (Georgopoulos et al. (1992) *Science* 258, 808; Georgopoulos et al. (1994) *Cell* 79, 143; Molnar et al. (1994) *Mol. Cell Biol.* 14, 8292; and Kaham et al. (1994) *Mol. Cell Biol.* 14, 7111). Ikaros mutant phenotypes suggest that this protein acts in concert with another protein with which it dimerizes. The Aiolos gene encodes a transcription factor which is homologous to Ikaros and can form dimers with it. In contrast to Ikaros which is expressed in pluripotent stem cells, Aiolos expression is first detected in committed lymphoid progenitors and increases as T and B cells mature. The expression patterns of Aiolos and Ikaros, the relative transcriptional activity of homo- and heterodimers of these proteins, and the dominant interfering effect of mutant Ikaros isoforms on the Aiolos activity suggest that Aiolos is an important regulator of lymphoid development. Thus, varying levels of Ikaros and Aiolos homodimers as well as heterodimers between these proteins modulate gene expression and regulate progression through the lymphoid lineages.

25 These examples are described in more detail herein.

Ikaros and Aiolos

30 The Ikaros gene encodes, by alternate splicing, a family of zinc finger transcription factors which are essential for development of the lymphopoietic system (Georgopoulos et al. (1992) *Science* 258, 808-812; Georgopoulos et al. (1994) *Cell* 79, 143-156; Molnar et al. (1994) *Mol. Cell Biol.* 14 8292-8303; and Hahm et al. (1994) *Mol. Cell Biol.* 14, 7111-7123). Ikaros expression is first detected in pluripotent hemopoietic stem cells and expression is maintained through all stages of lymphoid development. Mice homozygous for a deletion of the region encoding the Ikaros DNA binding domain lack committed progenitors as well as mature T and B lymphocytes

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and natural killer cells. (Georgopoulos et al. (1994) *Cell* 79, 143-156). In addition to this apparent role in the early development of lymphoid progenitors, Ikaros is also required for later events during T cell maturation (Winandy et al. (1995) *Cell* 83, 289-299). Mice heterozygous for this Ikaros mutation generate T cells which proliferate abnormally. They develop lymphoproliferative disorders and ultimately die of T cell leukemias and lymphomas.

The Ikaros protein isoforms all share a common C-terminal domain containing two zinc fingers to which different combinations of N-terminal zinc fingers are appended. The N-terminal zinc fingers are required for sequence specific DNA binding while the C-terminal zinc fingers mediate homo- and heterodimerization among the Ikaros isoforms (Molnar et al. (1994) *Mol. Cell. Biol.* 14 8292-8303. Homo- and heterodimerization of isoforms which contain a DNA-binding domain greatly increases their affinity for DNA and their transcriptional activity. Heterodimers containing one isoform which lacks a DNA binding domain are transcriptionally inert. Hence such isoforms can interfere with the activity of Ikaros isoforms which contain a DNA binding domain in a dominant negative fashion.

The C-terminal domain shared by all of the Ikaros isoforms was targeted by deletion in the mouse germ line. Mice homozygous for this mutation display a phenotype which is less severe than that caused by deletion of the DNA binding domain. The C-terminal Ikaros mutant mice lack most lymphocytes and NK cells but they do develop $\alpha\beta$ T cells. The milder phenotype may be due to a low level of activity retained in the proteins generated by the C-terminal Ikaros mutant allele. Alternatively, the C-terminal mutation could be the equivalent of a null for Ikaros activity while the more severe phenotype of the N-terminal deletion mutant may be explained by a dominant interfering effect of the Ikaros isoforms produced by the mutant allele on the activity of some other protein which is also required for commitment to and differentiation of the $\alpha\beta$ T lineage. The dominant negative influence of these proteins on other Ikaros isoforms with an intact DNA binding domain has been demonstrated by in vitro and in vivo assays. Since the zinc fingers in the Ikaros C-terminal domain display strong homology to the C-terminal zinc fingers of the *Drosophila* suppressor protein Hunchback (Tautz et al. (1987) *Nature* 327, 383) it appears that this domain existed prior to the expansion of the vertebrate genome and may be included in other proteins as well. Such proteins would have the potential to interact with Ikaros proteins when co-expressed and would be candidate targets for the dominant negative activity of the truncated Ikaros isoforms.

Degenerate oligonucleotides were used to amplify the C-terminal zinc finger domain from the mouse genome. Among the genes identified was Aiolos, a homolog of Ikaros whose expression is restricted to lymphoid lineage. The Aiolos protein shows extensive homology to the largest Ikaros isoform, Ik-1, throughout the DNA binding and C-terminal domains and can form homodimers and heterodimers with the Ikaros proteins. Aiolos homodimers are potent transcriptional activators while heterodimers between Aiolos and different Ikaros isoforms range in activity from slightly less potent to transcriptionally inert. Unlike Ikaros, Aiolos is not expressed in the hematopoietic stem cell compartment. Its expression is first detected at low levels in

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lymphoid progenitors and is strongly upregulated at the stage when rearrangement of T and B antigen receptors occurs. Thus, heterodimers of Aiolos and Ikaros are essential for the normal maturation of lymphocytes. The profound effects of the Ikaros DNA binding mutation reflect interference with the normal activity of both Aiolos and Ikaros during lymphocyte development.

5 Cloning of the Aiolos cDNA

In order to identify Ikaros homologs, degenerate primers were constructed to the sequences conserved between mouse Ikaros and Drosophila hunchback proteins (PCR primers: **Deg 3** TAC/TACCATC/TCACATGGGCTG/ACCA (SEQ ID NO:3) starting at residue 1278 of SEQ ID NO:1 and **Deg 4** G/ACCA/GCACATGTTG/ACACTC/TG/AAA (SEQ ID NO:4) starting at residue 1339 of SEQ ID NO:1. PCR was performed on chicken genomic DNA and products of the expected size (61 bp) were purified on a low melt agarose gel and subcloned into PCR2 vector (Invitrogen). Nucleotide sequence demonstrated that these clones fell into three classes. Phage containing the genomic sequence encoding these fragments were isolated from a genomic DNA library and the regions flanking the amplified fragments were sequenced. Analysis of this sequence demonstrated that one class of the clones represented the chicken homologue of Ikaros, while a second class represented the corresponding exon from a highly homologous gene, designated Aiolos (Fig. 2). Aiolos cDNA was isolated from a mouse spleen cDNA library using a probe spanning residues 796-1156 of SEQ ID NO:1. Clones isolated from this library fall into three classes representing alternative RNAs derived from Aiolos gene (Fig. 4). The corresponding genomic region was isolated by hybridization to probes spanning residues 1-650 and 796-1156 of SEQ ID NO:1. The mouse Aiolos cDNA nucleotide and corresponding amino acid sequence is given in Fig. 1.

15 Isolation of human Aiolos

Partial human Aiolos cDNAs were isolated by PCR amplification using mouse Aiolos primers Aio C (SEQ ID NO:5) and Aio A (SEQ ID NO:6), which are in mouse Aiolos exons 2 and 7, respectively. The nucleotide sequence of the longest of these cDNAs and the deduced amino acid sequence are presented in Figure 5 and correspond to SEQ ID NO:7 and SEQ ID NO:8, respectively. The sequence does not include the primers used for the amplification.

20 Isolation of Aiolos cDNA from Other Species

One of ordinary skill in the art can apply routine methods to obtain Aiolos cDNA from yet other species. The experiments described above outline isolation of Aiolos cDNA from mouse, chicken, and human. The Aiolos cDNA can be isolated from other species, e.g., from bovine, by methods analogous to those described above. For example, the bovine Aiolos cDNA can be isolated by probing a bovine spleen or thymus cDNA or genomic library with a probe homologous to mouse or human Aiolos cDNA described above.

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Alternative splice forms of Aiolos

PCR was used to determine whether alternative splice forms of Aiolos exist. Primer combinations AioC/AioA, Aio4F/AioA, and Aio5F/AioA were used to examine the possibility of alternate splicing of the Aiolos mRNA. AioC anneals within exon 3, Aio4F within
 5 exon 4, Aio5F within exon 5, and AioA within exon 7. The primer sequences are the following:

AioC GTG TGC GGG TTA TCC TGC ATT AGC (SEQ ID NO:5)
 AioF GTA ACC TCC TCC GTC ATA TTA AAC (SEQ ID NO:9)
 Aio5F CGA GCT TTT CTT CAG AAC CCT GAC (SEQ ID NO:10)
 10 AioA ATC GAA GCA GTG CCG CTT CTC ACC (SEQ ID NO:6)

Isoforms lacking exon 6 have been identified to date at a low abundance.

Functional domains are conserved between Aiolos and Ikaros proteins

Aiolos cDNA contains an open reading frame of 1521 nucleotides encoding a 58
 15 KD protein with 70% similarity to Ikaros (Fig. 6).

The general structure of Aiolos and Ikaros proteins is very similar, and four blocks of sequence are particularly well conserved. The first block of conservation encodes the zinc finger modules contained in the Ik-1 isoform which mediate DNA binding of the Ikaros protein (Molnar et al. (1994) *Mol. Cell. Biol.* 14 8292-8303). The second block of
 20 conservation has not been characterized functionally. The third block of conservation is a domain required for transcriptional activation by Ikaros (this domain is boxed in Figure 6). The fourth block of conservation corresponds to the zinc fingers which mediate dimerization.

Antibodies generated against two Aiolos peptides (amino acids 1-124 and amino
 25 acids 275-448) indicate that Aiolos polypeptide is approximately the same size as Ik-1 protein, i.e., approximately 57 kDa in size.

The structure and function of the Aiolos zinc finger domains are homologous with the zinc finger domains of Ikaros. Aiolos has four C terminal domains which mediate the binding of Aiolos to DNA and two C terminal regions which mediate the formation of
 30 Aiolos dimers.

Two highly conserved C-terminal Zn finger motifs mediate interactions between Aiolos and Ikaros proteins

The ability of the Aiolos zinc finger domain to engage in protein interactions was tested in a yeast two hybrid assay (Zervos et al. (1993) *Cell* 72, 223; and Gyuris et al.
 35 (1993) *Cell* 75, 1).

Segments of 500 nucleotides of the Aiolos or Ikaros cDNAs encoding the C-terminal 149 and 154 amino acids of these proteins, respectively, were inserted in the bait vector pLex202 to create in frame fusions with the LexA DNA binding domain (Ik-500

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and Aio-500, respectively). The B42 transcriptional activation domain in the pGJ prey vector was fused in frame to the full length Ikaros and Aiolos proteins as well as the following fragments of the cDNAs: the first five coding exons of Ik-1(Ik-N); the 500 nucleotides segments used to construct the bait constructs (Aio-500 and Ik-500); the entire coding sequence of the C-terminal exon of Aiolos (Aio-800) encoding a 232 amino acid long sequence; the full length Ikaros protein with point mutations in either the penultimate (M1) or ultimate (M2) zinc fingers, or both (M1 + M2). Combinations of Aiolos and Ikaros bait and prey vectors were transformed into the EGY48 yeast strain. EGY48 (MATa *trp1 ura3 his3 LEU2:pLexAop6-LEU2*) has a Leu2 gene as well as the pJK103 plasmid harboring the lacZ gene under the control of two high affinity ColE1 LexA operators maintained under Ura3 selection. Growth of yeast cells on Ura⁻ His⁻ Trp⁻ Leu⁻-galactose plates and color development on Ura⁻ His⁻ Trp⁻ -X-gal-galactose plates were used to score Aiolos and Ikaros protein interactions. Interactions between Aiolos and Ikaros baits and preys in the yeast two hybrid system result in the transcription of β -galactosidase and the production of blue colonies on X-gal indicator plates. Strong interactions between prey and bait recombinant proteins result in expression of both the Leu-2 and β -galactosidase genes.

The results are presented in Table I. The rate at which transformed yeast colonies turn blue on indicator plates suggests that the affinities of Aiolos for itself and for Ikaros protein are similar (+++). White colonies indicate a lack of interaction (-). A domain in the Aiolos protein that contains the last two Krüppel-like zinc fingers (Aio-500) interacts with itself either as an isolated domain (Aio-500, Aio-800) or in the context of the full length protein (Aiolos). Similar interactions were observed with the analogous Ikaros domain (Ik-500), either alone or in the context of the full length protein (Ikaros). Mutations in the Ikaros zinc finger motifs (M1, M2 and M1+M2) which abrogate Ikaros dimerization also abrogated Aiolos-Ikaros protein interactions. In contrast to the C-terminal fingers, the N-terminal finger motifs (Ik-N) were not capable of mediating such protein interactions. PJG is the prey vector used as a negative control. In a similar fashion, the equivalent Ikaros bait (154 aminoacids in size), Ik-500, interacted with recombinant prey proteins that contained either the C-terminal domain of Aiolos or Ikaros or the full length proteins. Ik-500 was, similarly to Aio-500, unable to interact with the interaction incompetent Ikaros mutants. In this assay, the affinities of Aiolos for itself or Ikaros were similar and indistinguishable to that of Ikaros for itself.

Table I

35

BAIT		
	Aiolos-500	Ikaros-500
PREY		
Aiolos	+++	+++

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Aio-500	+++	+++
Aio-800	+++	+++
Ikaros	+++	+++
Ik-500	+++	+++
Ik-N	-	-
Ikaros M1	-	-
Ikaros M2	-	-
Ikaros M1 + M2	-	-
pJG	-	-

Thus, this example shows that the C-terminal zinc fingers of Aiolos and Ikaros mediate protein dimerizations and that Aiolos and Ikaros can homodimerize and heterodimerize.

5 Aiolos and Ikaros heterodimerize in vivo

Heterodimers of Aiolos and Ikaros proteins were observed in transfected mammalian cells. Heterodimerization was shown by coimmunoprecipitations of the two proteins and by showing that both proteins localize to the same region in a cell.

Interactions between Aiolos and Ikaros proteins were confirmed by
 10 coimmunoprecipitations. Aiolos-(Flag) protein (10) and Ikaros protein (Ik-1), or a mutant Ikaros protein having point mutations in the zinc finger domain which prevents Ikaros homodimerization (IkM) were expressed in the epithelial cell line 293T and immunoprecipitated using an antibody to the Flag epitope (6, Eastman Kodak).
 Immunoprecipitates were run on a 10% SDS gel and analyzed by Western blotting with an
 15 Ikaros antibody. No Ikaros was observed in immunoprecipitates from untransfected controls. To confirm the levels of Ikaros and Aiolos protein produced in the transfected cells, Westerns on total protein were performed with the Ikaros and Flag antibodies. Similar amounts of Ik-1 or IkM and Aiolos proteins were produced in the transfected cell populations.

20 The results indicate that Ikaros protein coprecipitates with Aiolos upon immunoprecipitation of Aiolos-(FLAG) with an antibody to the tagged Aiolos protein. However, the dimerization mutant IkM was not coprecipitated with Aiolos-(FLAG). Thus, these results indicate that Aiolos and Ikaros heterodimerize *in vivo*.

Aiolos and Ikaros also co-localize in the nucleus of cells. Subcellular localization
 25 of Aiolos protein was determined upon its expression in NIH-3T3 fibroblasts. NIH-3T3 fibroblasts were transfected with one or more of expression vectors encoding Aiolos-(FLAG), Ikaros Ik-1 or Ik-6. The Ik-6 isoform of Ikaros lacks a DNA binding domain and is normally found in the cytoplasm. The FLAG epitope was detected with a the same anti-FLAG monoclonal antibody described above and a secondary goat anti-mouse IgG
 30 antibody conjugated to rhodamine (Boehringer Mannheim). NIH-3T3 fibroblasts transfected with Aiolos and Ikaros expression vectors were stained with anti-FLAG and

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rhodamine conjugated goat anti-mouse and with anti-Ikaros and goat anti-rabbit IgG FITC sequentially. No crossreactivity between preadsorbed secondary antibodies was detected. Cells were counterstained with hoechst 33258 for one hour in PBS at 1 µg/ml.

The results show that the Aiolos protein, tagged with the FLAG epitope (Hopp et al. (1988) *Biotech* 6, 1204-1210) is found in the nucleus when expressed in fibroblast cells. Immunofluorescence staining for either Aiolos or Ikaros proteins revealed a punctuate pattern of staining similar to that observed with polycomb proteins, some splicing factors, and the GATA proteins (Messmer et al. (1992) *Genes & Dev* 6, 1241-1254; Colwill et al (1996) *EMBO J* 15, 65-275; and Elefanty et al. (1996) *EMBO J* 15, 319-333). When Aiolos is coexpressed with an Ikaros isoform that is localized in the nucleus, e.g., Ik-1, both proteins are detected within the same region of the nucleus. In fact, the red and green signals of the labels generate a yellow signal, confirming the co-localization of these proteins. Interestingly, when Aiolos is coexpressed with an Ikaros isoform that is localized in the cytoplasm, e.g., Ik-6, both proteins co-localize to the nucleus.

Conserved function of the N-terminal zinc finger DNA binding domain in Aiolos and Ikaros proteins

Contacts between DNA and the alpha helical region in the C-terminal half of Kruppel-like zinc fingers are important in determining the sequence specificity of these interactions (Lee et al. (1989) *Science* 245, 635 and Pavletich et al. (1993) *Science* 261: 1701). The regions that bind DNA are perfectly conserved between Aiolos and Ikaros (Fig. 6). This example demonstrates that both proteins are capable of binding the same DNA sequences.

DNA binding assays (EMSA) were performed essentially as described in Molnar et al. (1994) *Mol. Cell. Biol.* 14, 8292-8303. GST-Aiolos and Ikaros fusion proteins and their GST fusion partner (0.5µg) were tested for binding to the IkBD1-TCAGCTTTTGGGAATACCCTGTCA (SEQ ID NO:11) oligonucleotide which contains a high affinity Ikaros binding site (100,000 cpm/reaction which equals 1 to 2 ngs of DNA). Competition assays were performed with Ik-BS1 and with Ik-BS8 TCAGCTTTTGGGggTACCCTGTCA (SEQ ID NO: 12) oligonucleotides used at 5-100 x molar excess.

The results of these binding assays show that high affinity complexes are formed between an Aiolos-GST fusion protein and an oligonucleotide containing a binding site for the Ik-1 protein. Hence Aiolos and Ikaros can, in principle, compete for similar binding sites in the genome.

Aiolos is a more potent transcriptional activator than Ikaros

Ikaros and Aiolos share a highly conserved 81 amino acid sequence which has been shown to mediate transcriptional activity of the Ikaros proteins. This activation domain of Ikaros is composed of a stretch of acidic amino acids followed by a stretch of hydrophobic residues, both of

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which are required for its full activation potential. This domain from Ikaros alone or the full length Ikaros protein confers transcriptional activity of a fusion protein with the LexA DNA binding domain. This example shows that the homologous domain in Aiolos is also a transcriptional activation domain in yeast and mammalian cells and that the Aiolos transcriptional activation domain provides stronger transcriptional activity than the homologous domain from Ikaros in mammalian cells.

The C- terminal domains of Aiolos and Ikaros were tested for their ability to activate transcription in yeast. For this example, expression constructs encoding the 232 and 149 C-terminal amino acids of Aiolos and fused to the LexA DNA binding domain were prepared, and termed Aio-800 and Aio-500, respectively. Expression constructs encoding the 232 and 154 most C-terminal residues of Ikaros fused to the LexA DNA binding domain were also prepared, and termed Ik-800 and Ik-500, respectively. These expression constructs were transformed into the EGY48 yeast strain. EGY48 (MATa *trp1 ura3 his3 LEU2:pLexAop6-LEU2*) has a *Leu2* gene as well as the pJK103 plasmid harboring the *lacZ* gene under the control of two high affinity ColEI LexA operators maintained under *Ura3* selection. The recombinant proteins were tested for their ability to activate the *Leu 2* gene and the *lacZ* genes using *Ura⁻ His⁻ Leu⁻ -glucose* and *Ura⁻ His⁻ Leu⁻ -X-gal-glucose* selections, respectively.

The results show that the 232 C-terminal amino acids of Aiolos fused to the LexA DNA binding domain activated strong expression of both the *Leu-2* and β -galactosidase genes in the yeast one hybrid system. No activity was detected with the 149 most C-terminal amino acids of Aiolos, which do not contain the conserved domain, in either assay. Thus, the protein domain in Aiolos, which is closely related in amino acid sequence to the transcriptional activation domain of Ikaros, is also capable of conferring transcriptional activation in yeast cells.

Although Aiolos and Ikaros display similar activities in yeast, Aiolos is a stronger activator in mammalian cells. In this example, Aiolos and the Ikaros isoforms Ik-1 and Ik-6 were co-transfected at different ratios together with the Ikaros-tkCAT reporter gene in NIH-3T3 cells as follows.

The ability of Aiolos homo- and Aiolos -Ikaros heterodimers to stimulate CAT activity from the Ikaros reporter plasmid 4xIk-BS1-tkCAT was determined in transient expression assays in NIH-3T3 fibroblast cells. NIH-3T3 cells in 100mm dish were co-transfected with the reporter plasmid 4xIk-BS1-tkCAT, containing 4 copies of a single high affinity Ikaros binding site or tkCAT (4 μ gs), with Aiolos and or Ikaros recombinant CDM8 expression vectors (5-15 μ gs) and with the pxGH5 (4 μ gs), a plasmid encoding the growth hormone which is used as an internal control of transfection. CDM8 was used to supplement amounts of expression vector DNA to 20 μ gs. Each transfection point was performed in triplicate or quadruplicate. 48 hours after transfection CAT and growth hormone (GH) assays were performed on cell lysates and supernatants respectively. Transfection efficiencies were normalized by growth hormone levels. Part of the cell pellet was lysed in protein sample buffer and used for Western analysis to

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determine Aiolos and Ikaros protein expression in transfected fibroblasts. The amount of protein was determined using Ikaros and Flag antibodies. The activities of Aiolos with or without the Flag epitope were indistinguishable in this assay. Co-transfections of the reporter plasmids with CDM8 vector alone were performed to establish the base level for CAT activity. Up to 5% variability was detected between transfections performed in triplicate.

The results are presented in Figure 7. Aiolos and Ikaros proteins were expressed at similar levels but the levels of CAT activity elicited by Aiolos were higher than those observed with Ik-1, the most potent activator of the Ikaros isoforms. In fact, Aiolos stimulated CAT activity by 25-50 fold, where Ik-1 elicited a 12-25 fold increase in expression in this assay. Co-expression of Ikaros and Aiolos proteins stimulated expression of the reporter gene to levels intermediate between those seen with Aiolos or Ikaros homodimers (e.g., compare Aiolos [10] versus Aiolos[5]+Ik-1[5] versus Ik-1 [10]).

Ikaros isoforms which lack a DNA binding domain interfere with the transcriptional activity of Aiolos proteins when both are expressed in the same cell (Figure 7, Aio + Ik-6). Similar results were obtained when Ikaros isoforms with and without a DNA binding domain were co-expressed. Hetero-dimers of the interfering Ikaros isoforms with other Ikaros proteins do not bind DNA. The dramatic decrease in Aiolos activity is most probably due to the formation of Aiolos-Ikaros heterodimers that do not bind DNA and therefore cannot activate transcription. Transfection with equimolar amounts of Aiolos and the Ik-6 isoform leads to the 65% reduction in CAT activity expected if Aiolos/Ik-6 heterodimers are transcriptionally inert. Addition of higher levels of Ik-6 further reduces transcription of the reporter gene. This effect is specific for the interfering isoform since addition of similar amounts of activating isoforms leads to a linear increase in transcriptional activity (Figure 7, Aio(5)+ Ik-1 (5)-(15)).

Therefore, Aiolos homodimers can compete with Ikaros homodimers for binding sites and can stimulate transcription to higher levels. The difference in activity of the two proteins can be accounted for by additional protein interactions that take place with a domain of the Ikaros proteins which is not conserved in Aiolos. Such protein interactions may specifically modulate the activity of Ikaros in mammalian cells during development without affecting Aiolos directly.

Aiolos expression is restricted to the lymphoid system

This example shows that in the adult mouse, Aiolos transcripts are detected exclusively in lymphoid tissues.

Total RNAs (10-20 µg) from thymus, spleen, bone marrow, brain, heart, kidney and liver of wild type mice and from bone marrow of mice homozygous for a mutation in the Ikaros DNA binding domain were used for Northern analysis. RNA purification and Northern analysis were performed as previously described (Georgopoulos et al. (1992) *Science* 258, 808-812). A 330 bp fragment derived from the last translated exon of Aiolos

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which does not cross-react with Ikaros sequences was used as a probe to detect Aiolos transcripts of 4.5 and 9 kb.

The results of the Northern blot hybridizations indicate that Aiolos expression levels are highest in the spleen, progressively lower in the thymus and bone marrow, and are undetectable in non-lymphoid tissues such as brain, heart, kidney or liver of a wild type mouse. The spleen is largely populated by mature B and T lymphocytes, while the majority of cells in the thymus are immature CD4+/CD8+ thymocytes which are in the process of rearranging their T antigen receptors. In the bone marrow, approximately 25% of the cells are pre-B cells at a stage of differentiation comparable to that of double positive thymocytes while the rest are predominantly erythroid and myeloid precursors (Hardy et al. (1991) *J. Exp. Med.* 173, 1213-1225). Aiolos mRNAs were not detected in the bone marrow of Ikaros mutant mice which is largely comprised of erythroid and myeloid cells and lacks detectable numbers of committed lymphoid precursors. These observations indicate that Aiolos is expressed in committed precursors of the B and T lineage and is upregulated upon their terminal differentiation.

Further information on Aiolos expression was obtained through *in situ* hybridization. Sections were prepared from E-12 to E-16 embryos as previously described (Georgopoulos et al. (1992) *Science* 258, 808-812). These were incubated with Ikaros or Aiolos specific ³²P-UTP RNA sense and antisense probes at 51°C for 12-16 hours. The Ikaros probe was 300 bp in size generated from the 3' untranslated region of its last exon. The Aiolos probe was generated from the first 330 bp of its last translated exon which show little homology to Ikaros sequences. Slides were washed with 0.5XSSC/0.1% SDS at 55°C and at 65°C, dehydrated and dipped in diluted photographic emulsion (NBT2). Dipped slides were exposed for 4 weeks, developed, stained with hematoxylin and eosin and analyzed by bright and dark field illumination on an Olympus microscope.

In situ hybridization to embryo sections indicated that Ikaros is expressed at the earliest stages of hemopoiesis, prior to the development of committed lymphoid precursors (Georgopoulos et al. (1992) *Science* 258, 808). It is found in the hemopoietic fetal liver at day 9.5 of gestation and in the thymus from the onset of its development. In contrast, Aiolos is not detected in the nervous system, hemopoietic liver and appears in the thymus only during the later stages of its development. This indicates that Aiolos is not expressed in hemopoietic stem cells, erythroid precursors, or in the lymphoid progenitors of epidermal $\gamma\delta$ T cells which predominate in the early thymus (Harvan et al. (1988) *Nature* 335, 443; Havran et al. (1990) *Nature* 344, 344; and Raulet et al. (1991) *Immunol Rev.* 120, 185). Expression in the late gestation thymus implies that Aiolos is found in double positive cells which are committed to the $\alpha\beta$ T cell lineage and are in the process of rearranging their T antigen receptor genes.

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To further characterize the relative expression of Ikaros and Aiolos during lymphocyte ontogeny, RNA from sorted lymphoid populations of wild type and mutant mice were analyzed by RT-PCR. cDNAs were prepared from FACS sorted populations isolated from the thymus, spleen, and bone marrow of wild type and mutant mice. cDNA yields were normalized to GAPDH concentrations using GAPDH primers. Aiolos and Ikaros cDNAs were amplified with gene specific primers derived from exons 3 and 7 and from exons 2 and 7, respectively, for 28 cycles. The Aiolos primers generate a single band and the Ikaros primers generate multiple bands corresponding to the alternatively spliced products of the Ikaros transcript (Georgopoulos et al. (1994) *Cell* 79, 143; and Molnar et al. (1994) *Mol. Cell Biol.* 14, 8292). Purification of the cells and RT PCR were performed essentially as set forth below.

Separation of purified cell populations were performed as follows. B220⁺ (pro-B, preB/B and B) and B220⁻ (T) populations were obtained from bone marrow and spleen of wild type C57BL/6 or RAG-1 ^{-/-} mice by magnetic cells sorting (Hardy et al. (1991) *J. Exp. Med.* 173, 1213-1225). First, lymphocytes were enriched by centrifugation of total bone marrow or spleen cells through a layer of Lymphocyte®-M (Cedarlane Laboratories, Hornby, Canada). The enriched lymphocytes were washed twice with cold PBS/BSA (PBS supplemented with 1% BSA, 5 mM EDTA and 0.01% sodium azide.), resuspended at a concentration of 10⁷ cells/ml in PBS/BSA, and incubated at 6° - 12°C for 15 minutes with anti-B220 MicroBeads (MACS). To monitor the purity of the the positively-selected cells and the flowthrough, fluorescein isothiocyanate (FITC) conjugated rat anti-B220 antibody was added and incubated for a further five minutes. B220⁺ cells were separated using a MACS magnetic separation column (Miltenyi Biotec GmbH). FACS analysis of the resulting B220⁺ and B220⁻ populations determined that these were 85-95% pure. Double positive and single positive thymic-cell populations were obtained by flow cytometry of cells from thymuses of wild type C57BL/6 mice. Thymic cells were incubated 30 minutes on ice with phycoerythrin (PE)- conjugated anti-CD4 and FITC-conjugated anti-CD8 antibodies (Pharmingen), after which they were washed and separated. using a Coulter sorter, into a single positive population, which included both CD4⁺CD8⁻ and CD4⁻CD8⁺ cells, and CD4⁺CD8⁺ double positive population. The single positive population was then further sorted into CD4⁺CD8⁻ and CD4⁻CD8⁺ populations.

Bone marrow cell suspensions were prepared from 8 to 12 week old C57BL/6J mice by gentle crushing of whole femurs and tibias in a ceramic mortar using PBS containing 2% heat inactivated fetal bovine serum (PBS/2% FBS). Cells were layered over Nycodenz with a density of 1.077 g/ml (Nycomed, Oslo, Norway) and centrifuged 30 minutes at 1000x g. The band of low density cells at the interface was removed, washed once in PBS/2% PBS. and resuspended in a cocktail of purified rat antibodies recognizing the lineage-specific antigens CD11b/MAC-1, CD45R/B220, Ly6G/Gr-1, CD4, CD8, and Ter119 (Pharmingen, San Diego, CA). After a 30 minute incubation on ice, the antibody-coated cells were removed by two rounds of

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immunomagnetic bead depletion on a Vario MACS BS column (Miltenyi Biotec, Sunnyvale, CA) using a 23G needle to restrict flow. The lineage-negative cells were then stained with FITC-conjugated D7 (anti-Sca-1) and PE-conjugated anti-c-kit (Pharmingen) for 30 minutes on ice, followed by one wash in PBS/2%FBS containing 2µg/ml propidium iodide (PI). Viable (PI-negative) cells were sorted on a FACStarPlus (Becton-Dickinson, San Jose, CA). Total RNA was prepared by homogenizing the samples (350µl maximum) using QIAshredder columns and RNeasy spin columns (Qiagen). Samples of 5×10^4 cells were processed and the RNA was eluted in DEPC-treated water in a final volume of 30µl. Two-color analysis of Sca-1 and c-kit revealed staining profiles identical to that reported by Okada et al., 1992. Based on these studies, Sca-1+c-kit (primitive repopulating stem cells) and Sca-1-c-kit+ (myeloid-committed progenitors) were sorted. Lineage negative cells were also stained with anti-Sca-1-FITC, anti-c-kit -PE and anti Sca-2-Red 613 and sorted into Sca-1⁺/Sca2^{-/lo}, Sca-1⁺/Sca-2^{dull} and Sca-1⁺/Sca-2^{bright}.

RT-PCR was performed as follows. Up to 5 µg of RNA were reverse transcribed in a total volume of 25 µl, which included 1X first strand buffer (Gibco-BRL), 4mM DTT, 150 ng random hexamer primers, 0.4 mM of each deoxynucleotide triphosphate, 1U Prime RNase inhibitor (5' ->3', Inc.) and 200 U Superscript II reverse transcriptase (Gibco-BRL). RNA and primers, in a total volume of 12 µl, were heated to 65°C for 10 mins before adding buffer, deoxynucleotides, DTT, RNase inhibitor, and reverse transcriptase. The reactions were incubated at 37°C for 45 minutes, followed by an incubation at 42°C for 45 minutes. Finally, 1 U RNase H (Gibco-BRL) was added, followed by an incubation at 37°C for 30 minutes. cDNAs were prepared from CD4⁺/CD8⁺ and CD4⁺, CD8⁺ sorted thymocytes, Rag-1^{-/-} thymocytes, B220⁺ cells from wild type bone marrow, B220⁺ cells from Rag-1^{-/-} bone marrow, B220⁺ and B220⁻ cells isolated from wild type spleen, Rag-1^{-/-} spleen, Ikaros^{-/-} bone marrow and spleen and from Sca1⁻/ckit⁺ and Sca1⁺/ckit⁺ stem cells populations. cDNA from each reaction was used directly for radiolabeled PCR. Reactions included up to 4µl of cDNA, 1X PCR reaction buffer (Boehringer-Mannheim), 0.1µg BSA, 100 ng each of 5' and 3' primers, 0.2 mM of each deoxynucleotide triphosphate, and 5 µCi each of [α -³²P]dATP and dCTP (3000 Ci/mmol) in a total volume of 50 µl. Primers specific for Ikaros, Ex2F and Ex7R have been previously described (Georgopoulos et al. (1994) *Cell* 79, 143-156). Primers specific for Aiolos were:

AioA: ATCGAAGCAGTGCCGCTTCTCACC (SEQ ID NO:6); and
AioC: GTGTGCGGGTTATCCTGCATTAGC (SEQ ID NO:5).

Primers specific for GAPDH were:

GAPDHF: ATGGTGAAGGTCGGTGTGAACGGATTTGGC (SEQ ID NO:13); and
GAPDHR: GCATCGAAGGTGGAAGAGTGGGAGTTGCTG (SEQ ID NO:14).

Amplification parameters consisted of 95°C for 5 minutes, 60°C for 5 minutes, at which point Taq polymerase (Boehringer-Mannheim) was added to each sample, followed by 27 cycles of 95°C for 15 seconds, 60°C for 20 seconds, and 72°C for 30 seconds. PCR products were

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visualized by electrophoresis through an 8% polyacrylamide - 1X TBE gel, followed by autoradiography of the dried gels.

The results indicate that Ikaros transcripts are readily detectable in the pluripotent stem cell population that can give rise to both lymphoid and myeloid/erythroid lineages (Sca-1⁺/c-kit⁺ (Van de Rijn et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 4634; and Okada et al. (1992) *Blood* 80, 3044). Ikaros transcripts were also found to be expressed at high levels in the more committed hemopoietic precursors (Sca-1⁺/c-kit⁺, mainly myeloid and erythroid precursors (Van de Rijn et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 4634; and Okada et al. (1992) *Blood* 80, 3044). In contrast, Aiolos expression was not readily detected in either of these heterogeneous populations. Low amounts of Aiolos were detected by prolonged exposure of the RT-PCR reactions in the multipotent progenitor population which is enriched for cells whose potential is restricted to the lymphoid lineages (Sca-1⁺/c-kit⁺/Sca-2⁺/lin⁻/lo⁽¹⁵⁾). Similar exposures failed to detect Aiolos in the pluripotent stem cell population. Low levels of Aiolos were also detected in the bone marrow of Ikaros mutant mice. These mice lack definitive lymphocyte precursors as well as more mature lymphoid cells, but the bone marrow may contain the most primitive lymphoid progenitors arrested in their differentiation. No expression of Aiolos was detected in the spleen of these mice upon prolonged exposure. Thus, in contrast to Ikaros, which is present in significant amounts from the early pluripotent stem cell stage, Aiolos is expressed only in cells which are committed to the lymphoid lineage.

Committed T cell progenitors progress from a double negative precursor through a double positive stage to the single positive thymocytes (Pearse et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 1614; and Godfrey et al. (1993) *Immunol Today* 14, 547). The double negative precursor thymocytes are rare in wild type mice. In Rag-1 deficient mice, which lack a component of the recombinase complex required for lymphocyte maturation, early B and T cell precursors are arrested in development and accumulate in the bone marrow and thymus respectively (Mobaerts, et al. (1992) *Cell* 68, 869; and Shinkai et al. (1992) *Cell* 68 855). Aiolos was barely detected in double negative pre-thymocytes isolated from the Rag-1 mutant thymus but moderate levels of Ikaros were expressed. However, Aiolos mRNA was readily detectable in immature double positive thymocytes and in the CD4 and CD8 single positive thymocytes derived from them.

In the B lineage, a similar pattern of Aiolos expression was observed. The pro-B cells isolated from Rag-1 deficient mice expressed Ikaros but very low amounts of Aiolos. Pre-B and B cells from wild type bone marrow expressed high levels of both Ikaros and Aiolos. Among cells sorted from the spleen, Aiolos was expressed at higher levels in B cells than in T cells, while Ikaros displayed the opposite pattern. Therefore, although Ikaros predominates during the early stages of T and B cell maturation, expression of

Aiolos increases significantly during the intermediate stages of the T and B lineage and comes to exceed that of Ikaros in mature B cells.

It is believed that natural killer (NK) cells are of lymphoid origin and share a common precursor with T lymphocytes (Hackett et al. (1986) *J Immunol.* 136, 3124; and Rodenwald et al. (1992) *Cell* 69, 139). Expression of Ikaros and Aiolos was examined in the spleen of Rag-1 deficient mice which is enriched for NK cells (Mobaerts, et al. (1992) *Cell* 68, 869; Shinkai et al. (1992) *Cell* 68 855; Hackett et al. (1986) *J Immunol.* 136, 3124; and Rodenwald et al. (1992) *Cell* 69, 139). Although Ikaros was abundantly expressed in Rag mutant splenocytes, significantly lower amounts of Aiolos were detected. In Ikaros mutant mice the spleen is populated by the non-lymphoid branch of the hemopoietic lineage (Georgepoulos et al. (1994) *Cell* 79, 143). Aiolos expression was not detected among these myeloid and erythroid cells.

Role of Aiolos and Ikaros homo- and hetero-dimers in lineage commitment and differentiation in the lymphoid lineages

The expression patterns of Ikaros and Aiolos indicates that variations in the relative levels of these proteins are important for the progression of a cell through the lymphoid lineage. A model of the role of these proteins in development of the lymphoid lineages is represented in Figure 8. Early in hemopoiesis, only Ikaros is expressed and Ikaros dimeric complexes are required and perhaps are sufficient to regulate the expression of genes that set the lymphoid fate in the differentiation of a pluripotent hemopoietic stem cell. Alternatively, interactions of Ikaros with yet undescribed and distinct factors may be required for commitment to the lymphoid lineages. As a consequence of these Ikaros mediated commitment events, Aiolos becomes expressed in primitive lymphoid progenitors and can form heterodimers with the Ikaros proteins. These Ikaros-Aiolos heterodimers are transcriptionally more active than Ikaros homodimers and may regulate the expression of genes that control the transition to definitive T and B lymphocyte precursors. As Aiolos is upregulated in pre-T (CD4⁺/CD8⁺) and pre-B(B220/Ig μ) cell precursors, the levels of Ikaros-Aiolos heterodimers increase and may allow for the later events in lymphocyte differentiation such as V to D-J and V-J rearrangement of immunoglobulin and TCR genes to take place (Hardy et al. (1993) *J. Exp. Med.* 178, 1213 and Li et al. *J. exp. Med.* 178, 951). Finally, in mature B cells where Aiolos expression predominates, transcriptionally potent Aiolos homodimers may control functions that are unique to these mature lymphocytes. Aiolos homodimers in mature T and B cells may be essential in regulating functions of these cells including gene expression events during their activation.

Therefore, normal progression through the T and B lineages may require the sequential expression of Ikaros-Ikaros, Ikaros-Aiolos and Aiolos-Aiolos dimeric complexes. Interference with Aiolos activity may affect lymphocyte maturation and function. In mice heterozygous for the DNA binding (dominant interfering) Ikaros

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mutation, defects in lymphocyte development are first observed in double positive thymocytes when Aiolos expression is normally upregulated. Since at this stage in differentiation Ikaros is expressed at higher levels than Aiolos, mutant Ikaros isoforms may readily sequester Aiolos proteins in inactive heterodimers which are unable to exert their function in T cell maturation. Although these dominant negative Ikaros isoforms are also expressed in B cells, defects in this mouse are limited to the T lineage. The different ratio of Aiolos to Ikaros mRNAs in B lymphocytes may result in insufficient mutant Ikaros proteins to titrate Aiolos and block its function in the B lineage.

Formation of transcriptionally potent Aiolos homodimers in developing thymocytes may also have adverse effects on their maturation. Although mice homozygous for a deletion of the Ikaros dimerization domain generate some $\alpha\beta$ T cells, these cells differentiate abnormally. The Ikaros isoforms generated by this mutation cannot dimerize and do not prevent Aiolos from forming homodimers. The defects observed in the T lineage are consistent with the activation of transcriptional programs normally found in later stages, perhaps as a consequence of premature accumulation of Aiolos homodimers.

These studies on Aiolos and Ikaros expression and function indicate that both members of this gene family act in concert to regulate lymphocyte differentiation. At the earliest stage of lymphoid lineage determination, Ikaros is the predominant regulator of target gene activity while Aiolos is expressed at very low levels. As a cell progresses through the lymphoid lineage, Aiolos is upregulated and its heterodimers with Ikaros proteins become important regulators of the transcriptional changes required for lymphocyte maturation. Finally in mature B cells, Aiolos homodimers predominate, while in cells of the T lineage Ikaros remains expressed at relatively higher levels. Aiolos and Ikaros dimeric complexes may also regulate the function of mature B and T lymphocytes during an immune response.

Transgenic animals

Aiolos knockouts with C terminal lesions (a deletions involving exons 3-5) were made. Aiolos knockouts with N terminal lesions (a deletions involving the 5' end of exon 7, which contains the dimerization domain) were also made. The former knockout is a dominant negative and is thought to interfere with DNA binding. It resulted in hyperproliferation of B cells and shows increased serum levels of IgE but are otherwise normal at 2-3 weeks of age. Fifty percent of B cells were IgE secretors, thus Aiolos appears to be involved in the Type I hyper acute response and in B cell regulation. The N terminal knockout homozygote produced no Aiolos protein, as determined by Western blotting.

Gene Therapy

The gene constructs of the invention can also be used as a part of a gene therapy protocol to deliver nucleic acids encoding either an agonistic or antagonistic form of an Aiolos polypeptide. The invention features expression vectors for *in vivo* transfection and expression of an Aiolos polypeptide in particular cell types (e.g., dermal cells) so as to

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reconstitute the function of, enhance the function of, or alternatively, antagonize the function of an Aiolos polypeptide in a cell in which the polypeptide is expressed or misexpressed.

5 Expression constructs of Aiolos polypeptide, may be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively delivering the Aiolos gene to cells *in vivo*. Approaches include insertion of the subject gene into viral vectors including recombinant retroviruses, adenovirus, adeno-associated virus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered with the help of, for
10 example, cationic liposomes (lipofectin) or derivatized (e.g. antibody conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or CaPO₄ precipitation carried out *in vivo*.

15 A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, e.g. a cDNA encoding an Aiolos polypeptide. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, e.g., by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

20 Retrovirus vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes *in vivo*, particularly into humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. The development of specialized cell lines (termed "packaging cells") which produce only replication-defective
25 retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood* 76, 271). A replication defective retrovirus can be packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for
30 infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are known to those skilled in the art. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic
35 retroviral systems include ψ Crip, ψ Cre, ψ 2 and ψ Am. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see for example Eglitis, et al. (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464; Wilson et al. (1988) *Proc.*

- Natl. Acad. Sci. USA* 85:3014-3018; Armentano et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6141-6145; Huber et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8039-8043; Ferry et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8377-8381; Chowdhury et al. (1991) *Science* 254:1802-1805; van Beusechem et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:7640-7644;
- 5 Kay et al. (1992) *Human Gene Therapy* 3:641-647; Dai et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:10892-10895; Hwu et al. (1993) *J. Immunol.* 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573).

- Another viral gene delivery system useful in the present invention utilizes
- 10 adenovirus-derived vectors. The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See, for example, Berkner et al. (1988) *BioTechniques* 6:616; Rosenfeld et al. (1991) *Science* 252:431-434; and Rosenfeld et al. (1992) *Cell* 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad
- 15 type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are known to those skilled in the art. Recombinant adenoviruses can be advantageous in certain circumstances in that they are not capable of infecting nondividing cells and can be used to infect a wide variety of cell types, including epithelial cells (Rosenfeld et al. (1992) cited *supra*).
- Furthermore, the virus particle is relatively stable and amenable to purification and
- 20 concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA).
- 25 Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner et al. cited *supra*; Haj-Ahmand and Graham (1986) *J. Virol.* 57:267).

- Yet another viral vector system useful for delivery of the subject Aiolos gene is the
- 30 adeno-associated virus (AAV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al. *Curr. Topics in Micro. and Immunol.* (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al. (1992) *Am. J. Respir. Cell. Mol. Biol.* 7:349-356;
- 35 Samulski et al. (1989) *J. Virol.* 63:3822-3828; and McLaughlin et al. (1989) *J. Virol.* 62:1963-1973). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as that described in Tratschin et al. (1985) *Mol. Cell. Biol.* 5:3251-3260 can be used to

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introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:6466-6470; Tratschin et al. (1985) *Mol. Cell. Biol.* 4:2072-2081; Wondisford et al. (1988) *Mol. Endocrinol.* 2:32-39; Tratschin et al. (1984) *J. Virol.* 51:611-619; and
5 Florte et al. (1993) *J. Biol. Chem.* 268:3781-3790).

In addition to viral transfer methods, such as those illustrated above, non-viral methods can also be employed to cause expression of an Aiolos polypeptide in the tissue of a mammal, such as a human. Most nonviral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of
10 macromolecules. In preferred embodiments, non-viral gene delivery systems of the present invention rely on endocytic pathways for the uptake of the subject Aiolos gene by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

In a representative embodiment, a gene encoding an Aiolos polypeptide can be
15 entrapped in liposomes bearing positive charges on their surface (e.g., lipofectins) and (optionally) which are tagged with antibodies against cell surface antigens of the target tissue (Mizuno et al. (1992) *No Shinkei Geka* 20:547-551; PCT publication WO91/06309; Japanese patent application 1047381; and European patent publication EP-A-43075).

In clinical settings, the gene delivery systems for the therapeutic Aiolos gene can be
20 introduced into a patient by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, e.g. by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional
25 regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent 5,328,470) or by stereotactic injection (e.g. Chen et al. (1994) *PNAS* 91: 3054-3057). In a preferred embodiment of the invention, the
30 Aiolos gene is targeted to hematopoietic cells.

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery system can be produced in tact from recombinant cells, e.g. retroviral vectors, the
35 pharmaceutical preparation can comprise one or more cells which produce the gene delivery system.

Antisense Therapy

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Another aspect of the invention relates to the use of the isolated nucleic acid in "antisense" therapy. As used herein, "antisense" therapy refers to administration or *in situ* generation of oligonucleotides or their derivatives which specifically hybridize (e.g. bind) under cellular conditions, with the cellular mRNA and/or genomic DNA encoding an Aiolos polypeptide, or mutant thereof, so as to inhibit expression of the encoded protein, e.g. by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, "antisense" therapy refers to the range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

In one embodiment, the antisense construct binds to a naturally-occurring sequence of an Aiolos gene which, for example, is involved in expression of the gene. These sequences include, for example, start codons, stop codons, and RNA primer binding sites.

In another embodiment, the antisense construct binds to a nucleotide sequence which is not present in the wild type gene. For example, the antisense construct can bind to a region of an Aiolos gene which contains an insertion of an exogenous, non-wild type sequence. Alternatively, the antisense construct can bind to a region of an Aiolos gene which has undergone a deletion, thereby bringing two regions of the gene together which are not normally positioned together and which, together, create a non-wild type sequence.

When administered *in vivo* to a subject, antisense constructs which bind to non-wild type sequences provide the advantage of inhibiting the expression of mutant Aiolos gene, without inhibiting expression of any wild type Aiolos gene.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular mRNA which encodes a Aiolos polypeptide. Alternatively, the antisense construct is an oligonucleotide probe which is generated *ex vivo* and which, when introduced into the cell causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences of an Aiolos gene. Such oligonucleotide probes are preferably modified oligonucleotide which are resistant to endogenous nucleases, e.g. exonucleases and/or endonucleases, and is therefore stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by Van der Krol et al. (1988) *Biotechniques* 6:958-976; and Stein et al. (1988) *Cancer Res* 48:2659-2668.

Accordingly, the modified oligomers of the invention are useful in therapeutic, diagnostic, and research contexts. In therapeutic applications, the oligomers are utilized in

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a manner appropriate for antisense therapy in general. For such therapy, the oligomers of the invention can be formulated for a variety of loads of administration, including systemic and topical or localized administration. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous for injection, the
5 oligomers of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the oligomers may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included in the invention.

The compounds can be administered orally, or by transmucosal or transdermal
10 means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives, and detergents. Transmucosal administration may be through nasal sprays or using suppositories. For oral administration, the oligomers are formulated into
15 conventional oral administration forms such as capsules, tablets, and tonics. For topical administration, the oligomers of the invention are formulated into ointments, salves, gels, or creams as known in the art.

In addition to use in therapy, the oligomers of the invention may be used as diagnostic reagents to detect the presence or absence of the target DNA or RNA sequences
20 to which they specifically bind.

The antisense constructs of the present invention, by antagonizing the expression of an Aiolos gene, can be used in the manipulation of tissue, both *in vivo* and in *ex vivo* tissue cultures.

Transgenic Animals

25 The invention includes transgenic animals which include cells (of that animal) which contain an Aiolos transgene and which preferably (though optionally) express (or misexpress) an endogenous or exogenous Aiolos gene in one or more cells in the animal.

The Aiolos transgene can encode a mutant Aiolos polypeptide. Such animals can be used as disease models or can be used to screen for agents effective at correcting the
30 misexpression of Aiolos. Alternatively, the Aiolos transgene can encode the wild-type forms of the protein, or can encode homologs thereof, including both agonists and antagonists, as well as antisense constructs. In preferred embodiments, the expression of the transgene is restricted to specific subsets of cells, or tissues utilizing, for example, cis-acting sequences that control expression in the desired pattern. Tissue-specific regulatory
35 sequences and conditional regulatory sequences can be used to control expression of the transgene in certain spatial patterns. Temporal patterns of expression can be provided by, for example, conditional recombination systems or prokaryotic transcriptional regulatory

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sequences. In preferred embodiments, the transgenic animal carries a "knockout" Aiolos gene, i.e., a deletion of all or a part of the Aiolos gene.

Genetic techniques which allow for the expression of transgenes, that are regulated *in vivo* via site-specific genetic manipulation, are known to those skilled in the art. For example, genetic systems are available which allow for the regulated expression of a recombinase that catalyzes the genetic recombination a target sequence. As used herein, the phrase "target sequence" refers to a nucleotide sequence that is genetically recombined by a recombinase. The target sequence is flanked by recombinase recognition sequences and is generally either excised or inverted in cells expressing recombinase activity.

10 Recombinase catalyzed recombination events can be designed such that recombination of the target sequence results in either the activation or repression of expression of the subject Aiolos gene. For example, excision of a target sequence which interferes with the expression of a recombinant Aiolos gene, such as one which encodes an agonistic homolog, can be designed to activate expression of that gene. This interference with expression of

15 the protein can result from a variety of mechanisms, such as spatial separation of the Aiolos gene from the promoter element or an internal stop codon.

Moreover, the transgene can be made so that the coding sequence of the gene is flanked with recombinase recognition sequences and is initially transfected into cells in a 3' to 5' orientation with respect to the promoter element. In such an instance, inversion of the

20 target sequence will reorient the subject gene by placing the 5' end of the coding sequence in an orientation with respect to the promoter element which allow for promoter driven transcriptional activation. See e.g., descriptions of the *cre/loxP* recombinase system of bacteriophage P1 (Lakso et al. (1992) *PNAS* 89:6232-6236; Orban et al. (1992) *PNAS* 89:6861-6865) or the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et

25 al. (1991) *Science* 251:1351-1355; PCT publication WO 92/15694). Genetic recombination of the target sequence is dependent on expression of the Cre recombinase. Expression of the recombinase can be regulated by promoter elements which are subject to regulatory control, e.g., tissue-specific, developmental stage-specific, inducible or repressible by externally added agents. This regulated control will result in genetic

30 recombination of the target sequence only in cells where recombinase expression is mediated by the promoter element. Thus, the activation expression of the recombinant Aiolos gene can be regulated via control of recombinase expression.

Similar conditional transgenes can be provided using prokaryotic promoter sequences which require prokaryotic proteins to be simultaneous expressed in order to

35 facilitate expression of the transgene. Exemplary promoters and the corresponding trans-activating prokaryotic proteins are given in U.S. Patent No. 4,833,080. Moreover, expression of the conditional transgenes can be induced by gene therapy-like methods wherein a gene encoding the trans-activating protein, e.g. a recombinase or a prokaryotic

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protein, is delivered to the tissue and caused to be expressed, such as in a cell-type specific manner. By this method, the Aiolos transgene could remain silent into adulthood until "turned on" by the introduction of the trans-activator.

Production of Fragments and Analogs

5 The inventor has provided the primary amino acid structure of an Aiolos polypeptide. Once an example of this core structure has been provided, one skilled in the art can alter the disclosed structure by producing fragments or analogs, and testing the newly produced structures for activity. Examples of prior art methods which allow the production and testing of fragments and analogs are discussed below. These, or analogous
10 methods can be used to make and screen fragments and analogs of an Aiolos polypeptide having at least one biological activity e.g., which react with an antibody (e.g., a monoclonal antibody) specific for an Aiolos polypeptide.

Generation of Fragments

15 Fragments of a protein can be produced in several ways, e.g., recombinantly, by proteolytic digestion, or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide. Expression of the mutagenized DNA produces polypeptide fragments. Digestion with "end-nibbling" endonucleases can thus generate DNA's which encode an
20 array of fragments. DNA's which encode fragments of a protein can also be generated by random shearing, restriction digestion or a combination of the above-discussed methods.

25 Fragments can also be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. For example, peptides of the present invention may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or divided into overlapping fragments of a desired length.

Production of Altered DNA and Peptide Sequences: Random Methods

30 Amino acid sequence variants of a protein can be prepared by random mutagenesis of DNA which encodes a protein or a particular domain or region of a protein. Useful methods include PCR mutagenesis and saturation mutagenesis. A library of random amino acid sequence variants can also be generated by the synthesis of a set of degenerate oligonucleotide sequences. (Methods for screening proteins in a library of variants are elsewhere herein.)

PCR Mutagenesis

35 In PCR mutagenesis, reduced Taq polymerase fidelity is used to introduce random mutations into a cloned fragment of DNA (Leung et al., 1989, *Technique* 1:11-15). This is a very powerful and relatively rapid method of introducing random mutations. The DNA region to be mutagenized is amplified using the polymerase chain reaction (PCR) under conditions that reduce the fidelity of DNA synthesis by Taq DNA polymerase, e.g., by

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using a dGTP/dATP ratio of five and adding Mn^{2+} to the PCR reaction. The pool of amplified DNA fragments are inserted into appropriate cloning vectors to provide random mutant libraries.

Saturation Mutagenesis

- 5 Saturation mutagenesis allows for the rapid introduction of a large number of single base substitutions into cloned DNA fragments (Mayers et al., 1985, *Science* 229:242). This technique includes generation of mutations, e.g., by chemical treatment or irradiation of single-stranded DNA *in vitro*, and synthesis of a complementary DNA strand. The mutation frequency can be modulated by modulating the severity of the treatment, and
- 10 essentially all possible base substitutions can be obtained. Because this procedure does not involve a genetic selection for mutant fragments both neutral substitutions, as well as those that alter function, are obtained. The distribution of point mutations is not biased toward conserved sequence elements.

Degenerate Oligonucleotides

- 15 A library of homologs can also be generated from a set of degenerate oligonucleotide sequences. Chemical synthesis of a degenerate sequences can be carried out in an automatic DNA synthesizer, and the synthetic genes then ligated into an appropriate expression vector. The synthesis of degenerate oligonucleotides is known in the art (see for example, Narang, SA (1983) *Tetrahedron* 39:3; Itakura et al. (1981)
- 20 *Recombinant DNA, Proc 3rd Cleveland Sympos. Macromolecules*, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477. Such techniques have been employed in the directed evolution of other proteins (see, for example, Scott et al. (1990) *Science* 249:386-390; Roberts et al. (1992) *PNAS* 89:2429-2433; Devlin et al.
- 25 (1990) *Science* 249: 404-406; Cwirla et al. (1990) *PNAS* 87: 6378-6382; as well as U.S. Patents Nos. 5,223,409, 5,198,346, and 5,096,815).

Production of Altered DNA and Peptide Sequences: Methods for Directed Mutagenesis

- 30 Non-random or directed, mutagenesis techniques can be used to provide specific sequences or mutations in specific regions. These techniques can be used to create variants which include, e.g., deletions, insertions, or substitutions, of residues of the known amino acid sequence of a protein. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conserved amino acids and then with more radical choices depending upon results achieved, (2) deleting the target residue, or (3) inserting residues of
- 35 the same or a different class adjacent to the located site, or combinations of options 1-3.

Alanine Scanning Mutagenesis

Alanine scanning mutagenesis is a useful method for identification of certain residues or regions of the desired protein that are preferred locations or domains for

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mutagenesis, Cunningham and Wells (*Science* 244:1081-1085, 1989). In alanine scanning, a residue or group of target residues are identified (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine). Replacement of an amino acid can affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions are then refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed desired protein subunit variants are screened for the optimal combination of desired activity.

Oligonucleotide-Mediated Mutagenesis

Oligonucleotide-mediated mutagenesis is a useful method for preparing substitution, deletion, and insertion variants of DNA, see, e.g., Adelman et al., (*DNA* 2:183, 1983). Briefly, the desired DNA is altered by hybridizing an oligonucleotide encoding a mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the desired protein. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the desired protein DNA. Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al. (*Proc. Natl. Acad. Sci. USA*, 75: 5765[1978]).

Cassette Mutagenesis

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al. (*Gene*, 34:315 [1985]). The starting material is a plasmid (or other vector) which includes the protein subunit DNA to be mutated. The codon(s) in the protein subunit DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the desired protein subunit DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is

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synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are comparable with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated desired protein subunit DNA sequence.

Combinatorial Mutagenesis

Combinatorial mutagenesis can also be used to generate mutants, e.g., a library of variants which is generated by combinatorial mutagenesis at the nucleic acid level, and is encoded by a variegated gene library. For example, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential sequences are expressible as individual peptides, or alternatively, as a set of larger fusion proteins containing the set of degenerate sequences.

Primary High-Through-Put Methods for Screening Libraries of Peptide Fragments or Homologs

Various techniques are known in the art for screening generated mutant gene products. Techniques for screening large gene libraries often include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the genes under conditions in which detection of a desired activity, e.g., in this case, binding to an antibody specific for a Aiolos polypeptide. Each of the techniques described below is amenable to high through-put analysis for screening large numbers of sequences created, e.g., by random mutagenesis techniques.

Display Libraries

In one approach to screening assays, the candidate peptides are displayed on the surface of a cell or viral particle, and the ability of particular cells or viral particles to bind an appropriate receptor protein via the displayed product is detected in a "panning assay". For example, the gene library can be cloned into the gene for a surface membrane protein of a bacterial cell, and the resulting fusion protein detected by panning (Ladner et al., WO 88/06630; Fuchs et al. (1991) *Bio/Technology* 9:1370-1371; and Goward et al. (1992) *TIBS* 18:136-140). In a similar fashion, a detectably labeled ligand can be used to score for potentially functional peptide homologs. Fluorescently labeled ligands, e.g., receptors, can be used to detect homolog which retain ligand-binding activity. The use of fluorescently labeled ligands, allows cells to be visually inspected and separated under a fluorescence microscope, or, where the morphology of the cell permits, to be separated by a fluorescence-activated cell sorter.

A gene library can be expressed as a fusion protein on the surface of a viral particle. For instance, in the filamentous phage system, foreign peptide sequences can be expressed on the surface of infectious phage, thereby conferring two significant benefits. First, since

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these phage can be applied to affinity matrices at concentrations well over 10^{13} phage per milliliter, a large number of phage can be screened at one time. Second, since each infectious phage displays a gene product on its surface, if a particular phage is recovered from an affinity matrix in low yield, the phage can be amplified by another round of infection. The group of almost identical *E. coli* filamentous phages M13, fd., and f1 are most often used in phage display libraries. Either of the phage gIII or gVIII coat proteins can be used to generate fusion proteins without disrupting the ultimate packaging of the viral particle. Foreign epitopes can be expressed at the NH₂-terminal end of pIII and phage bearing such epitopes recovered from a large excess of phage lacking this epitope (Ladner et al. PCT publication WO 90/02909; Garrard et al., PCT publication WO 92/09690; Marks et al. (1992) *J. Biol. Chem.* 267:16007-16010; Griffiths et al. (1993) *EMBO J* 12:725-734; Clackson et al. (1991) *Nature* 352:624-628; and Barbas et al. (1992) *PNAS* 89:4457-4461).

A common approach uses the maltose receptor of *E. coli* (the outer membrane protein, LamB) as a peptide fusion partner (Charbit et al. (1986) *EMBO* 5, 3029-3037). Oligonucleotides have been inserted into plasmids encoding the LamB gene to produce peptides fused into one of the extracellular loops of the protein. These peptides are available for binding to ligands, e.g., to antibodies, and can elicit an immune response when the cells are administered to animals. Other cell surface proteins, e.g., OmpA (Schorr et al. (1991) *Vaccines* 91, pp. 387-392), PhoE (Agterberg, et al. (1990) *Gene* 88, 37-45), and PAL (Fuchs et al. (1991) *Bio/Tech* 9, 1369-1372), as well as large bacterial surface structures have served as vehicles for peptide display. Peptides can be fused to pilin, a protein which polymerizes to form the pilus-a conduit for interbacterial exchange of genetic information (Thiry et al. (1989) *Appl. Environ. Microbiol.* 55, 984-993). Because of its role in interacting with other cells, the pilus provides a useful support for the presentation of peptides to the extracellular environment. Another large surface structure used for peptide display is the bacterial motive organ, the flagellum. Fusion of peptides to the subunit protein flagellin offers a dense array of many peptides copies on the host cells (Kuwajima et al. (1988) *Bio/Tech.* 6, 1080-1083). Surface proteins of other bacterial species have also served as peptide fusion partners. Examples include the *Staphylococcus* protein A and the outer membrane protease IgA of *Neisseria* (Hansson et al. (1992) *J. Bacteriol.* 174, 4239-4245 and Klauser et al. (1990) *EMBO J.* 9, 1991-1999).

In the filamentous phage systems and the LamB system described above, the physical link between the peptide and its encoding DNA occurs by the containment of the DNA within a particle (cell or phage) that carries the peptide on its surface. Capturing the peptide captures the particle and the DNA within. An alternative scheme uses the DNA-binding protein LacI to form a link between peptide and DNA (Cull et al. (1992) *PNAS USA* 89:1865-1869). This system uses a plasmid containing the LacI gene with an oligonucleotide cloning site at its 3'-end. Under the controlled induction by arabinose, a

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LacI-peptide fusion protein is produced. This fusion retains the natural ability of LacI to bind to a short DNA sequence known as LacO operator (LacO). By installing two copies of LacO on the expression plasmid, the LacI-peptide fusion binds tightly to the plasmid that encoded it. Because the plasmids in each cell contain only a single oligonucleotide
5 sequence and each cell expresses only a single peptide sequence, the peptides become specifically and stably associated with the DNA sequence that directed its synthesis. The cells of the library are gently lysed and the peptide-DNA complexes are exposed to a matrix of immobilized receptor to recover the complexes containing active peptides. The associated plasmid DNA is then reintroduced into cells for amplification and DNA
10 sequencing to determine the identity of the peptide ligands. As a demonstration of the practical utility of the method, a large random library of dodecapeptides was made and selected on a monoclonal antibody raised against the opioid peptide dynorphin B. A cohort of peptides was recovered, all related by a consensus sequence corresponding to a six-residue portion of dynorphin B. (Cull et al. (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89:1869)

15 This scheme, sometimes referred to as peptides-on-plasmids, differs in two important ways from the phage display methods. First, the peptides are attached to the C-terminus of the fusion protein, resulting in the display of the library members as peptides having free carboxy termini. Both of the filamentous phage coat proteins, pIII and pVIII, are anchored to the phage through their C-termini, and the guest peptides are placed into the
20 outward-extending N-terminal domains. In some designs, the phage-displayed peptides are presented right at the amino terminus of the fusion protein. (Cwirla, et al. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87, 6378-6382) A second difference is the set of biological biases affecting the population of peptides actually present in the libraries. The LacI fusion molecules are confined to the cytoplasm of the host cells. The phage coat fusions are
25 exposed briefly to the cytoplasm during translation but are rapidly secreted through the inner membrane into the periplasmic compartment, remaining anchored in the membrane by their C-terminal hydrophobic domains, with the N-termini, containing the peptides, protruding into the periplasm while awaiting assembly into phage particles. The peptides in the LacI and phage libraries may differ significantly as a result of their exposure to
30 different proteolytic activities. The phage coat proteins require transport across the inner membrane and signal peptidase processing as a prelude to incorporation into phage. Certain peptides exert a deleterious effect on these processes and are underrepresented in the libraries (Gallop et al. (1994) *J. Med. Chem.* 37(9):1233-1251). These particular biases are not a factor in the LacI display system.

35 The number of small peptides available in recombinant random libraries is enormous. Libraries of 10^7 - 10^9 independent clones are routinely prepared. Libraries as large as 10^{11} recombinants have been created, but this size approaches the practical limit for clone libraries. This limitation in library size occurs at the step of transforming the

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DNA containing randomized segments into the host bacterial cells. To circumvent this limitation, an *in vitro* system based on the display of nascent peptides in polysome complexes has recently been developed. This display library method has the potential of producing libraries 3-6 orders of magnitude larger than the currently available phage/phagemid or plasmid libraries. Furthermore, the construction of the libraries, expression of the peptides, and screening, is done in an entirely cell-free format.

In one application of this method (Gallop et al. (1994) *J. Med. Chem.* 37(9):1233-1251), a molecular DNA library encoding 10^{12} decapeptides was constructed and the library expressed in an *E. coli* S30 *in vitro* coupled transcription/translation system. Conditions were chosen to stall the ribosomes on the mRNA, causing the accumulation of a substantial proportion of the RNA in polysomes and yielding complexes containing nascent peptides still linked to their encoding RNA. The polysomes are sufficiently robust to be affinity purified on immobilized receptors in much the same way as the more conventional recombinant peptide display libraries are screened. RNA from the bound complexes is recovered, converted to cDNA, and amplified by PCR to produce a template for the next round of synthesis and screening. The polysome display method can be coupled to the phage display system. Following several rounds of screening, cDNA from the enriched pool of polysomes was cloned into a phagemid vector. This vector serves as both a peptide expression vector, displaying peptides fused to the coat proteins, and as a DNA sequencing vector for peptide identification. By expressing the polysome-derived peptides on phage, one can either continue the affinity selection procedure in this format or assay the peptides on individual clones for binding activity in a phage ELISA, or for binding specificity in a completion phage ELISA (Barret, et al. (1992) *Anal. Biochem* 204,357-364). To identify the sequences of the active peptides one sequences the DNA produced by the phagemid host.

Secondary Screens

The high through-put assays described above can be followed by secondary screens in order to identify further biological activities which will, e.g., allow one skilled in the art to differentiate agonists from antagonists. The type of a secondary screen used will depend on the desired activity that needs to be tested. For example, an assay can be developed in which the ability to inhibit an interaction between a protein of interest and its respective ligand can be used to identify antagonists from a group of peptide fragments isolated though one of the primary screens described above.

Therefore, methods for generating fragments and analogs and testing them for activity are known in the art. Once the core sequence of a protein of interest is identified, such as the primary amino acid sequence of Aiolos polypeptide as disclosed herein, it is routine to perform for one skilled in the art to obtain analogs and fragments.

Peptide Analogs of Aiolos

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Peptide analogs of an Aiolos polypeptide are preferably less than 400, 300, 200, 150, 130, 110, 90, 70 amino acids in length, preferably less than 50 amino acids in length, most preferably less than 30, 20 or 10 amino acids in length. In preferred embodiments, the peptide analogs of an Aiolos polypeptide are at least about 10, 20, 30, 50, 100 or 130 amino acids in length.

Peptide analogs of an Aiolos polypeptide have preferably at least about 60%, 70%, 80%, 85%, 90%, 95% or 99% homology or sequence similarity with the naturally occurring Aiolos polypeptide.

Peptide analogs of an Aiolos polypeptide differ from the naturally occurring Aiolos polypeptide by at least 1, 2, 5, 10 or 20 amino acid residues; preferably, however, they differ in less than 15, 10 or 5 amino acid residues from the naturally occurring Aiolos polypeptide.

Useful analogs of an Aiolos polypeptide can be agonists or antagonists. Antagonists of an Aiolos polypeptide can be molecules which form the Aiolos-Ikaros dimers but which lack some additional biological activity such as transcriptional activation of genes that control lymphocyte development. Aiolos antagonists and agonists are derivatives which can modulate, e.g., inhibit or promote, lymphocyte maturation and function.

A number of important functional Aiolos domains have been identified by the inventors. This body of knowledge provides guidance for one skilled in the art to make Aiolos analogs. One would expect nonconservative amino acid changes made in a domain to disrupt activities in which that domain is involved. Conservative amino acid changes, especially those outside the important functional domains, are less likely to modulate a change in activity. A discussion of conservative amino acid substitutions is provided herein.

The general structure of Aiolos and Ikaros proteins is very similar, and four blocks of sequence are particularly well conserved. The first block of conservation encodes the zinc finger modules contained in the Ik-1 isoform which mediate DNA binding of the Ikaros protein (Molnar et al. (1994) *Mol. Cell. Biol.* 14 8292-8303). The second block of conservation has not been characterized functionally.

The third block of conservation is a highly conserved 81 amino acid sequence which has been shown to mediate transcriptional activity of the Ikaros proteins (this domain is boxed in Figure 6). This activation domain of Ikaros is composed of a stretch of acidic amino acids followed by a stretch of hydrophobic residues, both of which are required for its full activation potential. This domain from Ikaros alone or the full length Ikaros protein confers transcriptional activity of a fusion protein with the LexA DNA binding domain. This example shows that the homologous domain in Aiolos is also a transcriptional activation domain in yeast and mammalian cells and that the Aiolos transcriptional

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activation domain provides stronger transcriptional activity than the homologous domain from Ikaros in mammalian cells. The results show that the 232 C-terminal amino acids of Aiolos is capable of conferring transcriptional activation in yeast cells. No activity was detected with the 149 most C-terminal amino acids of Aiolos, which do not contain the conserved domain.

The fourth block of conservation corresponds to the zinc fingers which mediate dimerization. A C-terminal 149 amino acids of Aiolos which contain the two terminal zinc finger domains mediate protein dimerization.

Antibodies

The invention also includes antibodies specifically reactive with a subject Aiolos polypeptide or Aiolos-Ikaros dimers. Anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (See, for example, *Antibodies: A Laboratory Manual* ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of the subject Aiolos polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies. In a preferred embodiment, the subject antibodies are immunospecific for antigenic determinants of the Aiolos-Ikaros dimers or Aiolos polypeptide of the invention, e.g. antigenic determinants of a polypeptide of SEQ ID NO:2 or SEQ ID NO:8.

The term "antibody", as used herein, intended to include fragments thereof which are also specifically reactive with an Aiolos polypeptide or Aiolos-Ikaros dimers. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')₂ fragments can be generated by treating antibody with pepsin. The resulting F(ab')₂ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

Both monoclonal and polyclonal antibodies (Ab) directed against Aiolos-Ikaros dimers or Aiolos polypeptides, or fragments or analogs thereof, and antibody fragments such as Fab' and F(ab')₂, can be used to block the action of an Aiolos and/or Ikaros polypeptide and allow the study of the role of an Aiolos polypeptide of the present invention.

Antibodies which specifically bind Aiolos-Ikaros dimers or Aiolos polypeptide epitopes can also be used in immunohistochemical staining of tissue samples in order to evaluate the abundance and pattern of expression of Aiolos-Ikaros dimer or Aiolos polypeptide. Anti-Aiolos polypeptide antibodies can be used diagnostically in immuno-

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precipitation and immuno-blotting to detect and evaluate wild type or mutant Aiolos polypeptide levels in tissue or bodily fluid as part of a clinical testing procedure. Likewise, the ability to monitor Aiolos-Ikaros dimer or Aiolos polypeptide levels in an individual can allow determination of the efficacy of a given treatment regimen for an individual afflicted
5 with disorders associated with modulation of lymphocyte differentiation and/or proliferation. The level of an Aiolos-Ikaros dimer or Aiolos polypeptide can be measured in tissue, such as produced by biopsy.

Another application of anti-Aiolos antibodies of the present invention is in the immunological screening of cDNA libraries constructed in expression vectors such as
10 λ gt11, λ gt18-23, λ ZAP, and λ ORF8. Messenger libraries of this type, having coding sequences inserted in the correct reading frame and orientation, can produce fusion proteins. For instance, λ gt11 will produce fusion proteins whose amino termini consist of β -galactosidase amino acid sequences and whose carboxy termini consist of a foreign polypeptide. Antigenic epitopes of a subject Aiolos polypeptide can then be detected with
15 antibodies, as, for example, reacting nitrocellulose filters lifted from infected plates with anti-Aiolos polypeptide antibodies. Phage, scored by this assay, can then be isolated from the infected plate. Thus, the presence of Aiolos homologs can be detected and cloned from other animals, and alternate isoforms (including splicing variants) can be detected and cloned from human sources.

20 Drug Screening Assays

By making available purified and recombinant-Aiolos polypeptides, the present invention provides assays which can be used to screen for drugs which are either agonists or antagonists of the normal cellular function, in this case, of the subject Aiolos polypeptide. In one embodiment, the assay evaluates the ability of a compound to
25 modulate binding between an Aiolos polypeptide and a naturally occurring ligand, e.g., an antibody specific for a Aiolos polypeptide or an Ikaros polypeptide. A variety of assay formats will suffice and, in light of the present inventions, will be comprehended by skilled artisan.

In many drug screening programs which test libraries of compounds and natural
30 extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test
35 compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an

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alteration of binding affinity with other proteins or change in enzymatic properties of the molecular target.

Other Embodiments

Included in the invention are: allelic variations; natural mutants; induced mutants; proteins encoded by DNA that hybridizes under high or low stringency conditions to a nucleic acids which encode polypeptides of SEQ ID NO:2 or SEQ ID NO:8 (for definitions of high and low stringency see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989, 6.3.1 - 6.3.6, hereby incorporated by reference); and, polypeptides specifically bound by antisera to an Aiolos polypeptide.

Nucleic acids and polypeptides of the invention includes those that differ from the sequences disclosed herein by virtue of sequencing errors in the disclosed sequences.

Also included in the invention is a composition which includes an Aiolos polypeptide, e.g., an Aiolos/Aiolos dimer or an Aiolos/Ikaros peptide, and one or more additional components, e.g., a carrier, diluent, or solvent. The additional component can be one which renders the composition useful for *in vitro*, *in vivo*, pharmaceutical, or veterinary use. Examples of *in vitro* use are binding studies. Examples of *in vivo* use are the induction of antibodies.

The invention also includes fragments, preferably biologically active fragments, or analogs of an Aiolos polypeptide. A biologically active fragment or analog is one having any *in vivo* or *in vitro* activity which is characteristic of the Aiolos polypeptide shown in SEQ ID NO:2 or SEQ ID NO:8, or of other naturally occurring Aiolos polypeptides, e.g., one or more of the biological activities described above. Especially preferred are fragments which exist *in vivo*, e.g., fragments which arise from post transcriptional processing or which arise from translation of alternatively spliced RNA's. Fragments include those expressed in native or endogenous cells, e.g., as a result of post-translational processing, e.g., as the result of the removal of an amino-terminal signal sequence, as well as those made in expression systems, e.g., in CHO cells. Because peptides, such as an Aiolos polypeptide, often exhibit a range of physiological properties and because such properties may be attributable to different portions of the molecule, a useful Aiolos polypeptide fragment or Aiolos polypeptide analog is one which exhibits a biological activity in any biological assay for Aiolos polypeptide activity. Most preferably the fragment or analog possesses 10%, preferably 40%, or at least 90% of the activity of an Aiolos polypeptide (SEQ ID NO:2 or SEQ ID NO:8), in any *in vivo* or *in vitro* Aiolos polypeptide activity assay.

Analog can differ from a naturally occurring Aiolos polypeptide in amino acid sequence or in ways that do not involve sequence, or both. Non-sequence modifications include *in vivo* or *in vitro* chemical derivatization of an Aiolos polypeptide. Non-sequence

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modifications include changes in acetylation, methylation, phosphorylation, carboxylation, or glycosylation.

- Preferred analogs include an Aiolos polypeptide (or biologically active fragments thereof) whose sequences differ from the wild-type sequence by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish the Aiolos polypeptide biological activity. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Other conservative substitutions can be taken from the table below.

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TABLE I
CONSERVATIVE AMINO ACID REPLACEMENTS

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, β -Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or L-I-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Other analogs within the invention are those with modifications which increase peptide stability; such analogs may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids; and cyclic analogs.

As used herein, the term "fragment", as applied to an Aiolos polypeptide analog, will ordinarily be at least about 20-residues, more typically at least about 40 residues, preferably at least about 60 residues in length. Fragments of an Aiolos polypeptide can be generated by methods known to those skilled in the art. The ability of a candidate fragment to exhibit a biological activity of an Aiolos polypeptide can be assessed by methods known to those skilled in the art, as described herein. Also included are Aiolos polypeptides containing residues that are not required for biological activity of the peptide or that result from alternative mRNA splicing or alternative protein processing events.

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In order to obtain an Aiolos polypeptide, an Aiolos polypeptide-encoding DNA can be introduced into an expression vector, the vector introduced into a cell suitable for expression of the desired protein, and the peptide recovered and purified, by prior art methods. Antibodies to the peptides and proteins can be made by immunizing an animal, e.g., a rabbit or mouse, and recovering anti-Aiolos polypeptide antibodies by prior art methods.

Equivalents

Those skilled in the art will be able to recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANTS: Katia Georgopoulos
Bruce Morgan
- (ii) TITLE OF INVENTION: The Aiolos Gene
- 10 (iii) NUMBER OF SEQUENCES: 22
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LAHIVE & COCKFIELD
(B) STREET: 60 State Street, Suite 510
15 (C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109-1875
- 20 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- 25 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
- 30 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Myers, Louis
(B) REGISTRATION NUMBER: 35,965
(C) REFERENCE/DOCKET NUMBER: MGP-042-2
- 35 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617)227-7400
(B) TELEFAX: (617)227-5941

(2) INFORMATION FOR SEQ ID NO:1:

- 40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1984 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
45 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
50 (A) NAME/KEY: CDS
(B) LOCATION: 374..1895
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- 55 CACGAGCGCA CACCGCTCGG CTCTCCTTGC GACACGCCCT CATCCCCGGT GTTCTCAAG 60
TAGACGTCCC GAGACGGTCG CTGAGGCACT GTTCCACGC GATCAGGGTT CCTCAGGCTT 120

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	GACATTCAAA AGTGGGTGCG GAACCCGCGG CACTCGGAGC GTGCTTTAAA GCGGCCGCCA	180
	GCCAGCGCCG CTCTAACCTC GCGCCCCGGC TGCCGGCGGC TCCCGCCCTG CATCTGCGCC	240
5	GACGCGACCG AGCGATCCCG GGGCCTCCCT GCGCCCGGAA TCTCCCGCCA GCCGCGCGGG	300
	TCCCCACGGC AGCAGCACGT GGAGCGGCCG CGGAGCCTGA GCGACAGCTG CAGCCCCGCG	360
10	GGCCCCGCGGC GAC ATG GAA GAT ATA CAA CCG ACT GTG GAG CTG AAA AGC Met Glu Asp Ile Gln Pro Thr Val Glu Leu Lys Ser	409
	1 5 10	
15	ACG GAG GAG CAG CCT CTG CCC ACA GAG AGC CCA GAC GCT CTG AAT GAC Thr Glu Glu Gln Pro Leu Pro Thr Glu Ser Pro Asp Ala Leu Asn Asp	457
	15 20 25	
20	TAC AGC TTG CCC AAA CCT CAT GAG ATA GAA AAC GTG GAC AGT AGA GAA Tyr Ser Leu Pro Lys Pro His Glu Ile Glu Asn Val Asp Ser Arg Glu	505
	30 35 40	
25	GCC CCA GCC AAT GAA GAC GAA GAT GCA GGA GAA GAT TCG ATG AAA GTG Ala Pro Ala Asn Glu Asp Glu Asp Ala Gly Glu Asp Ser Met Lys Val	553
	45 50 55 60	
30	AAA GAT GAA TAC AGC GAC AGA GAT GAG AAC ATT ATG AAG CCG GAG CCC Lys Asp Glu Tyr Ser Asp Arg Asp Glu Asn Ile Met Lys Pro Glu Pro	601
	65 70 75	
35	ATG GGA GAT GCA GAA GAG AGT GAA ATG CCT TAC AGC TAT GCA AGA GAA Met Gly Asp Ala Glu Glu Ser Glu Met Pro Tyr Ser Tyr Ala Arg Glu	649
	80 85 90	
40	TAC AGC GAC TAT GAA AGC ATT AAG CTG GAG AGA CAC GTG CCC TAT GAC Tyr Ser Asp Tyr Glu Ser Ile Lys Leu Glu Arg His Val Pro Tyr Asp	697
	95 100 105	
45	AAC AGC AGA CCA ACC AGT GGG AAG ATG AAC TGC GAC GTG TGC GGG TTA Asn Ser Arg Pro Thr Ser Gly Lys Met Asn Cys Asp Val Cys Gly Leu	745
	110 115 120	
50	TCC TGC ATT AGC TTC AAC GTC TTG ATG GTT CAT AAG CGA AGC CAT ACC Ser Cys Ile Ser Phe Asn Val Leu Met Val His Lys Arg Ser His Thr	793
	125 130 135 140	
55	GGC GAA CGC CCG TTC CAG TGT AAT CAG TGC GGG GCA TCT TTT ACT CAG Gly Glu Arg Pro Phe Gln Cys Asn Gln Cys Gly Ala Ser Phe Thr Gln	841
	145 150 155	
60	AAA GGT AAC CTC CTC CGT CAT ATT AAA CTG CAC ACG GGG GAA AAA CCT Lys Gly Asn Leu Leu Arg His Ile Lys Leu His Thr Gly Glu Lys Pro	889
	160 165 170	
65	TTT AAG TGT CAC CTC TGC AAC TAC GCA TGC CAA AGG AGA GAT GCG CTC Phe Lys Cys His Leu Cys Asn Tyr Ala Cys Gln Arg Arg Asp Ala Leu	937
	175 180 185	
70	ACG GGA CAC CTT AGG ACA CAT TCT GTG GAG AAG CCG TAC AAG TGT GAG Thr Gly His Leu Arg Thr His Ser Val Glu Lys Pro Tyr Lys Cys Glu	985

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	190	195	200	
5	TTC TGC GGA AGA AGC TAC AAG CAG AGA AGC TCC CTG GAG GAG CAC AAG Phe Cys Gly Arg Ser Tyr Lys Gln Arg Ser Ser Leu Glu Glu His Lys 205 210 215 220	1033		
10	GAA CGC TGC CGA GCT TTT CTT CAG AAC CCT GAC CTG GGG GAC GCT GCA Glu Arg Cys Arg Ala Phe Leu Gln Asn Pro Asp Leu Gly Asp Ala Ala 225 230 235	1081		
15	AGT GTG GAG GCA AGA CAC ATC AAA GCC GAG ATG GGA AGT GAG AGA GCT Ser Val Glu Ala Arg His Ile Lys Ala Glu Met Gly Ser Glu Arg Ala 240 245 250	1129		
20	CTC GTC CTG GAC AGA TTA GCA AGC AAT GTG GCT AAG CGA AAA AGC TCG Leu Val Leu Asp Arg Leu Ala Ser Asn Val Ala Lys Arg Lys Ser Ser 255 260 265	1177		
25	ATG CCT CAG AAA TTC ATC GGT GAG AAG CGG CAC TGC TTC GAT GCC AAC Met Pro Gln Lys Phe Ile Gly Glu Lys Arg His Cys Phe Asp Ala Asn 270 275 280	1225		
30	TAC AAT CCC GGC TAC ATG TAC GAG AAG GAG AAC GAG ATG ATG CAG ACC Tyr Asn Pro Gly Tyr Met Tyr Glu Lys Glu Asn Glu Met Met Gln Thr 285 290 295 300	1273		
35	CGG ATG ATG GAC CAA GCC ATC AAT AAC GCC ATC AGC TAT CTA GGG GCT Arg Met Met Asp Gln Ala Ile Asn Asn Ala Ile Ser Tyr Leu Gly Ala 305 310 315	1321		
40	GAA GCC TTC CGC CCC TTA GTC CAG ACT CCG CCT GCT CCC ACC TCT GAG Glu Ala Phe Arg Pro Leu Val Gln Thr Pro Pro Ala Pro Thr Ser Glu 320 325 330	1369		
45	ATG GTC CCA GTC ATC AGC AGT GTG TAC CCC ATA GCA CTT ACT CGG GCC Met Val Pro Val Ile Ser Ser Val Tyr Pro Ile Ala Leu Thr Arg Ala 335 340 345	1417		
50	GAT ATG CCA ATG GGG GCC CCG CAG GAG ATG GAA AAG AAA CGG ATC CTC Asp Met Pro Met Gly Ala Pro Gln Glu Met Glu Lys Lys Arg Ile Leu 350 355 360	1465		
55	CTG CCA GAG AAG ATC TTG CCT TCT GAA CGA GGT CTG TCC CCC AAT AAC Leu Pro Glu Lys Ile Leu Pro Ser Glu Arg Gly Leu Ser Pro Asn Asn 365 370 375 380	1513		
60	AGT GCC CAG GAC TCC ACA GAC ACC GAC AGC AAC CAC GAG GAT CGC CAA Ser Ala Gln Asp Ser Thr Asp Thr Asp Ser Asn His Glu Asp Arg Gln 385 390 395	1561		
65	CAT CTC TAC CAG CAA AGC CAC GTG GTC CTC CCC CAG GCC CGC AAT GGG His Leu Tyr Gln Gln Ser His Val Val Leu Pro Gln Ala Arg Asn Gly 400 405 410	1609		
70	ATG CCT CTT CTG AAG GAG GTC CCT CGC TCT TTT GAA CTC CTC AAG CCC Met Pro Leu Leu Lys Glu Val Pro Arg Ser Phe Glu Leu Leu Lys Pro 415 420 425	1657		

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CCT CCC ATC TGC CTG AGG GAC TCC ATC AAA GTG ATC AAC AAA GAA GGG 1705
 Pro Pro Ile Cys Leu Arg Asp Ser Ile Lys Val Ile Asn Lys Glu Gly
 430 435 440

5 GAG GTG ATG GAT GTG TTT CGA TGT GAC CAC TGC CAC GTC CTC TTC CTA 1753
 Glu Val Met Asp Val Phe Arg Cys Asp His Cys His Val Leu Phe Leu
 445 450 455 460

10 GAT TAT GTG ATG TTC ACC ATC CAC ATG GGG TGC CAT GGT TTC CGT GAT 1801
 Asp Tyr Val Met Phe Thr Ile His Met Gly Cys His Gly Phe Arg Asp
 465 470 475

15 CCC TTT GAG TGT AAC ATG TGT GGC TAT CGA AGC CAC GAT CGC TAT GAG 1849
 Pro Phe Glu Cys Asn Met Cys Gly Tyr Arg Ser His Asp Arg Tyr Glu
 480 485 490

TTC TCC TCT CAC ATC GCC AGA GGA GAG CAC AGA GCC ATG TTG AAG T 1895
 Phe Ser Ser His Ile Ala Arg Gly Glu His Arg Ala Met Leu Lys
 495 500 505

20 GAGCATCTGT CCTCAATGCG AGGGTCAACA TTGTTTTTTA AAGCTGATGG TAGCCTTATC 1955
 CAGTAGACTG AACTCAAACC CACCTCGAG 1984

25 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 507 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

35 Met Glu Asp Ile Gln Pro Thr Val Glu Leu Lys Ser Thr Glu Glu Gln
 1 5 10 15

40 Pro Leu Pro Thr Glu Ser Pro Asp Ala Leu Asn Asp Tyr Ser Leu Pro
 20 25 30

Lys Pro His Glu Ile Glu Asn Val Asp Ser Arg Glu Ala Pro Ala Asn
 35 40 45

45 Glu Asp Glu Asp Ala Gly Glu Asp Ser Met Lys Val Lys Asp Glu Tyr
 50 55 60

Ser Asp Arg Asp Glu Asn Ile Met Lys Pro Glu Pro Met Gly Asp Ala
 65 70 75 80

50 Glu Glu Ser Glu Met Pro Tyr Ser Tyr Ala Arg Glu Tyr Ser Asp Tyr
 85 90 95

Glu Ser Ile Lys Leu Glu Arg His Val Pro Tyr Asp Asn Ser Arg Pro
 100 105 110

55 Thr Ser Gly Lys Met Asn Cys Asp Val Cys Gly Leu Ser Cys Ile Ser
 115 120 125

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Phe Asn Val Leu Met Val His Lys Arg Ser His Thr Gly Glu Arg Pro
 130 135 140

5 Phe Gln Cys Asn Gln Cys Gly Ala Ser Phe Thr Gln Lys Gly Asn Leu
 145 150 155 160

Leu Arg His Ile Lys Leu His Thr Gly Glu Lys Pro Phe Lys Cys His
 165 170 175

10 Leu Cys Asn Tyr Ala Cys Gln Arg Arg Asp Ala Leu Thr Gly His Leu
 180 185 190

Arg Thr His Ser Val Glu Lys Pro Tyr Lys Cys Glu Phe Cys Gly Arg
 195 200 205

Ser Tyr Lys Gln Arg Ser Ser Leu Glu Glu His Lys Glu Arg Cys Arg
 210 215 220

20 Ala Phe Leu Gln Asn Pro Asp Leu Gly Asp Ala Ala Ser Val Glu Ala
 225 230 235 240

Arg His Ile Lys Ala Glu Met Gly Ser Glu Arg Ala Leu Val Leu Asp
 245 250 255

25 Arg Leu Ala Ser Asn Val Ala Lys Arg Lys Ser Ser Met Pro Gln Lys
 260 265 270

Phe Ile Gly Glu Lys Arg His Cys Phe Asp Ala Asn Tyr Asn Pro Gly
 275 280 285

Tyr Met Tyr Glu Lys Glu Asn Glu Met Met Gln Thr Arg Met Met Asp
 290 295 300

35 Gln Ala Ile Asn Asn Ala Ile Ser Tyr Leu Gly Ala Glu Ala Phe Arg
 305 310 315 320

Pro Leu Val Gln Thr Pro Pro Ala Pro Thr Ser Glu Met Val Pro Val
 325 330 335

40 Ile Ser Ser Val Tyr Pro Ile Ala Leu Thr Arg Ala Asp Met Pro Met
 340 345 350

Gly Ala Pro Gln Glu Met Glu Lys Lys Arg Ile Leu Leu Pro Glu Lys
 355 360 365

45 Ile Leu Pro Ser Glu Arg Gly Leu Ser Pro Asn Asn Ser Ala Gln Asp
 370 375 380

50 Ser Thr Asp Thr Asp Ser Asn His Glu Asp Arg Gln His Leu Tyr Gln
 385 390 395 400

Gln Ser His Val Val Leu Pro Gln Ala Arg Asn Gly Met Pro Leu Leu
 405 410 415

55 Lys Glu Val Pro Arg Ser Phe Glu Leu Leu Lys Pro Pro Pro Ile Cys
 420 425 430

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Leu Arg Asp Ser Ile Lys Val Ile Asn Lys Glu Gly Glu Val Met Asp
 435 440 445

5 Val Phe Arg Cys Asp His Cys His Val Leu Phe Leu Asp Tyr Val Met
 450 455 460

Phe Thr Ile His Met Gly Cys His Gly Phe Arg Asp Pro Phe Glu Cys
 465 470 475 480

10 Asn Met Cys Gly Tyr Arg Ser His Asp Arg Tyr Glu Phe Ser Ser His
 485 490 495

Ile Ala Arg Gly Glu His Arg Ala Met Leu Lys
 500 505

15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TACTACCATC TCACATGGGC TGACCA

26

30 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GACCAGCACA TGTTGACACT CTGAAA

26

45 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGTGCGGGT TATCCTGCAT TAGC

24

(2) INFORMATION FOR SEQ ID NO:6:

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
5 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATCGAAGCAG TGCCGCTTCT CACC 24
- (2) INFORMATION FOR SEQ ID NO:7:
15
(2) INFORMATION FOR SEQ ID NO:8:

(2) INFORMATION FOR SEQ ID NO:9:
- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

30 GTAACCTCCT CCGTCATATT AAAC 24
- (2) INFORMATION FOR SEQ ID NO:10:
- (i) SEQUENCE CHARACTERISTICS:
35 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGAGCTTTTC TTCAGAACCC TGAC 24
- 45 (2) INFORMATION FOR SEQ ID NO:11:
- (i) SEQUENCE CHARACTERISTICS:
50 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCAGCTTTTG GGAATACCCT GTCA 24

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(2) INFORMATION FOR SEQ ID NO:12:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

15 TCAGCTTTTG GGGGTACCCT GTCA 24

(2) INFORMATION FOR SEQ ID NO:13:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

30 ATGGTGAAGG TCGGTGTGAA CGGATTTGGC 30

(2) INFORMATION FOR SEQ ID NO:14:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCATCGAAGG TGGAAGAGTG GGAGTTGCTG 30

(2) INFORMATION FOR SEQ ID NO:15:

45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1788 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

55 (A) NAME/KEY: CDS
(B) LOCATION: 223..1515

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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	AATTCGTTCT ACCTTCTCTG AACCCAGTG GTGTGTCAAG GCCGGACTGG GAGCTTGGGG	60
	GAAGAGGAAG AGGAAGAGGA ATCTGCGGCT CATCCAGGGA TCAGGGTCCT TCCCAAGTGG	120
5	CCACTCAGAG GGGACTCAGA GCAAGTCTAG ATTTGTGTGG CAGAGAGAGA CAGCTCTCGT	180
	TTGGCCTTGG GGAGGCACAA GTCTGTTGAT AACCTGAAGA CA	222
10	ATG GAT GTC GAT GAG GGT CAA GAC ATG TCC CAA GTT TCA GGA AAG GAG Met Asp Val Asp Glu Gly Gln Asp Met Ser Gln Val Ser Gly Lys Glu	270
	1 5 10 15	
	AGC CCC CCA GTC AGT GAC ACT CCA GAT GAA GGG GAT GAG CCC ATG CCT	318
15	Ser Pro Pro Val Ser Asp Thr Pro Asp Glu Gly Asp Glu Pro Met Pro	
	20 25 30	
	GTC CCT GAG GAC CTG TCC ACT ACC TCT GGA GCA CAG CAG AAC TCC AAG	366
20	Val Pro Glu Asp Leu Ser Thr Thr Ser Gly Ala Gln Gln Asn Ser Lys	
	35 40 45	
	AGT GAT CGA GGC ATG GGT GAA CGG CCT TTC CAG TGC AAC CAG TCT GGG	414
	Ser Asp Arg Gly Met Gly Gln Arg Pro Phe Gln Cys Asn Gln Ser Gly	
	50 55 60	
25	GCC TCC TTT ACC CAG AAA GGC AAC CTC CTG CGG CAC ATC AAG CTG CAC	462
	Ala Ser Phe Thr Gln Lys Gly Asn Leu Leu Arg His Ile Lys Leu His	
	65 70 75 80	
30	TCG GGT GAG AAG CCC TTC AAA TGC CAT CTT TGC AAC TAT GCC TGC CGC	510
	Ser Gly Glu Lys Pro Phe Lys Cys His Leu Cys Asn Tyr Ala Cys Arg	
	85 90 95	
	CGG AGG GAC GCC CTC ACC GGC CAC CTG AGG ACG CAC TCC GTT GGT AAG	558
35	Arg Arg Asp Ala Leu Thr Gly His Leu Arg Thr His Ser Val Gly Lys	
	100 105 110	
	CCT CAC AAA TGT GGA TAT TGT GGC CGG AGC TAT AAA CAG CGA AGC TCT	606
40	Pro His Lys Cys Gly Tyr Cys Gly Arg Ser Tyr Lys Gln Arg Ser Ser	
	115 120 125	
	TTA GAG GAG CAT AAA GAG CGA TGC CAC AAC TAC TTG GAA AGC ATG GGC	654
	Leu Glu Glu His Lys Glu Arg Cys His Asn Tyr Leu Glu Ser Met Gly	
	130 135 140	
45	CTT CCG GGC GTG TGC CCA GTC ATT AAG GAA GAA ACT AAC CAC AAC GAG	702
	Leu Pro Gly Val Cys Pro Val Ile Lys Glu Glu Thr Asn His Asn Glu	
	145 150 155 160	
50	ATG GCA GAA GAC CTG TGC AAG ATA GGA GCA GAG AGG TCC CTT GTC CTG	750
	Met Ala Glu Asp Leu Cys Lys Ile Gly Ala Glu Arg Ser Leu Val Leu	
	165 170 175	
	GAC AGG CTG GCA AGC AAT GTC GCC AAA CGT AAG AGC TCT ATG CCT CAG	798
55	Asp Arg Leu Ala Ser Asn Val Ala Lys Arg Lys Ser Ser Met Pro Gln	
	180 185 190	
	AAA TTT CTT GGA GAC AAG TGC CTG TCA GAC ATG CCC TAT GAC AGT GCC	846

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	Lys	Phe	Leu	Gly	Asp	Lys	Cys	Leu	Ser	Asp	Met	Pro	Tyr	Asp	Ser	Ala	
			195					200					205				
5	AAC	TAT	GAG	AAG	GAG	GAT	ATG	ATG	ACA	TCC	CAC	GTG	ATG	GAC	CAG	GCC	894
	Asn	Tyr	Glu	Lys	Glu	Asp	Met	Met	Thr	Ser	His	Val	Met	Asp	Gln	Ala	
			210				215					220					
10	ATC	AAC	AAT	GCC	ATC	AAC	TAC	CTG	GGG	GCT	GAG	TCC	CTG	CGC	CCA	TTG	942
	Ile	Asn	Asn	Ala	Ile	Asn	Tyr	Leu	Gly	Ala	Glu	Ser	Leu	Arg	Pro	Leu	
			225			230					235					240	
15	GTG	CAG	ACA	CCC	CCC	GGT	AGC	TCC	GAG	GTG	GTG	CCA	GTC	ATC	AGC	TCC	990
	Val	Gln	Thr	Pro	Pro	Gly	Ser	Ser	Glu	Val	Val	Pro	Val	Ile	Ser	Ser	
				245					250						255		
	ATG	TAC	CAG	CTG	CAC	AAG	CCC	CCC	TCA	GAT	GGC	CCC	CCA	CGG	TCC	AAC	1038
	Met	Tyr	Gln	Leu	His	Lys	Pro	Pro	Ser	Asp	Gly	Pro	Pro	Arg	Ser	Asn	
				260					265					270			
20	CAT	TCA	GCA	CAG	GAC	GCC	GTG	GAT	AAC	TTG	CTG	CTG	CTG	TCC	AAG	GCC	1086
	His	Ser	Ala	Gln	Asp	Ala	Val	Asp	Asn	Leu	Leu	Leu	Leu	Ser	Lys	Ala	
			275					280						285			
25	AAG	TCT	GTG	TCA	TCG	GAG	CGA	GAG	GCC	TCC	CCG	AGC	AAC	AGC	TGC	CAA	1134
	Lys	Ser	Val	Ser	Ser	Glu	Arg	Glu	Ala	Ser	Pro	Ser	Asn	Ser	Cys	Gln	
			290				295					300					
30	GAC	TCC	ACA	GAT	ACA	GAG	AGC	AAC	GCG	GAG	GAA	CAG	CGC	AGC	GGC	CTT	1182
	Asp	Ser	Thr	Asp	Thr	Glu	Ser	Asn	Ala	Glu	Glu	Gln	Arg	Ser	Gly	Leu	
						310					315					320	
35	ATC	TAC	CTA	ACC	AAC	CAC	ATC	AAC	CCG	CAT	GCA	CGC	AAT	GGG	CTG	GCT	1230
	Ile	Tyr	Leu	Thr	Asn	His	Ile	Asn	Pro	His	Ala	Arg	Asn	Gly	Leu	Ala	
					325				330					335			
	CTC	AAG	GAG	GAG	CAG	CGC	GCC	TAC	GAG	GTG	CTG	AGG	GCG	GCC	TCA	GAG	1278
	Leu	Lys	Glu	Glu	Gln	Arg	Ala	Tyr	Glu	Val	Leu	Arg	Ala	Ala	Ser	Glu	
				340					345					350			
40	AAC	TCG	CAG	GAT	GCC	TTC	CGT	GTG	GTC	AGC	ACG	AGT	GGC	GAG	CAG	CTG	1326
	Asn	Ser	Gln	Asp	Ala	Phe	Arg	Val	Val	Ser	Thr	Ser	Gly	Glu	Gln	Leu	
			355					360					365				
45	AAG	GTG	TAC	AAG	TGC	GAA	CAC	TGC	CGC	GTG	CTC	TTC	CTG	GAT	CAC	GTC	1374
	Lys	Val	Tyr	Lys	Cys	Glu	His	Cys	Arg	Val	Leu	Phe	Leu	Asp	His	Val	
			370				375					380					
50	ATG	TAT	ACC	ATT	CAC	ATG	GGC	TGC	CAT	GGC	TGC	CAT	GGC	TTT	CGG	GAT	1422
	Met	Tyr	Thr	Ile	His	Met	Gly	Cys	His	Gly	Cys	His	Gly	Phe	Arg	Asp	
			385			390				395						400	
55	CCC	TTT	GAG	TGT	AAC	ATG	TGT	GGT	TAT	CAC	AGC	CAG	GAC	AGG	TAC	GAG	1470
	Pro	Phe	Glu	Cys	Asn	Met	Cys	Gly	Tyr	His	Ser	Gln	Asp	Arg	Tyr	Glu	
				405					410					415			
	TTC	TCA	TCC	CAT	ATC	ACG	CGG	GGG	GAG	CAT	CGT	TAC	CAC	CTG	AGC		1515
	Phe	Ser	Ser	His	Ile	Thr	Arg	Gly	Glu	His	Arg	Tyr	His	Leu	Ser		
				420				425						430			

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TAAACCCAGC CAGGCCCCAC TGAAGCACAA AGATAGCTGG TTATGCCTCC TTCCCGGCAG 1575

CTGGACCCAC AGCGGACAAT GTGGGAGTGG ATTTGCAGGC AGCATTTGTT CTTTATGTT 1635

5 GGTGTTTGG CGTTTCATT GCGTTGGAAG ATAAGTTTTT AATGTTAGTG ACAGGATTGC 1695

ATTGCATCAG CAACATTCAC AACATCCATC CTTCTAGCCA GTTTGTTCCTA CTGGTAGCTG 1755

10 AGGTTTCCCG GATATGTGGC TTCCTAACAC TCT 1788

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 1386 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1386

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AAT GTT AAA GTA GAG ACT CAG AGT GAT GAA GAG AAT GGG CGT GCC TGT 48

Asn Val Lys Val Glu Thr Gln Ser Asp Glu Glu Asn Gly Arg Ala Cys

30 1 5 10 15

GAA ATG AAT GGG GAA GAA TGT GCG GAG GAT TTA CGA ATG CTT GAT GCC 96

Glu Met Asn Gly Glu Glu Cys Ala Glu Asp Leu Arg Met Leu Asp Ala

20 25 30

35 TCG GGA GAG AAA ATG AAT GGC TCC CAC AGG GAC CAA GGC AGC TCG GCT 144

Ser Gly Glu Lys Met Asn Gly Ser His Arg Asp Gln Gly Ser Ser Ala

35 40 45

40 TTG TCG GGA GTT GGA GGC ATT CGA CTT CCT AAC GGA AAA CTA AAG TGT 192

Leu Ser Gly Val Gly Gly Ile Arg Leu Pro Asn Gly Lys Leu Lys Cys

50 55 60

GAT ATC TGT GGG ATC ATT TGC ATC GGG CCC AAT GTG CTC ATG GTT CAC 240

Asp Ile Cys Gly Ile Ile Cys Ile Gly Pro Asn Val Leu Met Val His

65 70 75 80

AAA AGA AGC CAC ACT GGA GAA CGG CCC TTC CAG TGC AAT CAG TGC GGG 288

Lys Arg Ser His Thr Gly Glu Arg Pro Phe Gln Cys Asn Gln Cys Gly

85 90 95

50 GCC TCA TTC ACC CAG AAG GGC AAC CTG CTC CGG CAC ATC AAG CTG CAT 336

Ala Ser Phe Thr Gln Lys Gly Asn Leu Leu Arg His Ile Lys Leu His

100 105 110

55 TCC GGG GAG AAG CCC TTC AAA TGC CAC CTC TGC AAC TAC GCC TGC CGC 384

Ser Gly Glu Lys Pro Phe Lys Cys His Leu Cys Asn Tyr Ala Cys Arg

115 120 125

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	CGG AGG GAC GCC CTC ACT GGC CAC CTG AGG ACG CAC TCC GTT GGT AAA	432
	Arg Arg Asp Ala Leu Thr Gly His Leu Arg Thr His Ser Val Gly Lys	
	130 135 140	
5	CCT CAC AAA TGT GGA TAT TGT GGC CGA AGC TAT AAA CAG CGA ACG TCT	480
	Pro His Lys Cys Gly Tyr Cys Gly Arg Ser Tyr Lys Gln Arg Thr Ser	
	145 150 155 160	
10	TTA GAG GAA CAT AAA GAG CGC TGC CAC AAC TAC TTG GAA AGC ATG GGC	528
	Leu Glu Glu His Lys Glu Arg Cys His Asn Tyr Leu Glu Ser Met Gly	
	165 170 175	
15	CTT CCG GGC ACA CTG TAC CCA GTC ATT AAA GAA GAA ACT AAG CAC AGT	576
	Leu Pro Gly Thr Leu Tyr Pro Val Ile Lys Glu Glu Thr Lys His Ser	
	180 185 190	
20	GAA ATG GCA GAA GAC CTG TGC AAG ATA GGA TCA GAG AGA TCT CTC GTG	624
	Glu Met Ala Glu Asp Leu Cys Lys Ile Gly Ser Glu Arg Ser Leu Val	
	195 200 205	
25	CTG GAC AGA CTA GCA AGT AAT GTC GCC AAA CGT AAG AGC TCT ATG CCT	672
	Leu Asp Arg Leu Ala Ser Asn Val Ala Lys Arg Lys Ser Ser Met Pro	
	210 215 220	
30	CAG AAA TTT CTT GGG GAC AAG GGC CTG TCC GAC ACG CCC TAC GAC AGT	720
	Gln Lys Phe Leu Gly Asp Lys Gly Leu Ser Asp Thr Pro Tyr Asp Ser	
	225 230 235 240	
35	GCC ACG TAC GAG AAG GAG AAC GAA ATG ATG AAG TCC CAC GTG ATG GAC	768
	Ala Thr Tyr Glu Lys Glu Asn Glu Met Met Lys Ser His Val Met Asp	
	245 250 255	
40	CAA GCC ATC AAC AAC GCC ATC AAC TAC CTG GGG GCC GAG TCC CTG CGC	816
	Gln Ala Ile Asn Asn Ala Ile Asn Tyr Leu Gly Ala Glu Ser Leu Arg	
	260 265 270	
45	CCG CTG GTG CAG ACG CCC CCG GGC GGT TCC GAG GTG GTC CCG GTC ATC	864
	Pro Leu Val Gln Thr Pro Pro Gly Gly Ser Glu Val Val Pro Val Ile	
	275 280 285	
50	AGC CCG ATG TAC CAG CTG CAC AGG CGC TCG GAG GGC ACC CCG CGC TCC	912
	Ser Pro Met Tyr Gln Leu His Arg Arg Ser Glu Gly Thr Pro Arg Ser	
	290 295 300	
55	AAC CAC TCG GCC CAG GAC AGC GCC GTG GAG TAC CTG CTG CTG CTC TCC	960
	Asn His Ser Ala Gln Asp Ser Ala Val Glu Tyr Leu Leu Leu Leu Ser	
	305 310 315 320	
60	AAG GCC AAG TTG GTG CCC TCG GAG CGC GAG GCG TCC CCG AGC AAC AGC	1008
	Lys Ala Lys Leu Val Pro Ser Glu Arg Glu Ala Ser Pro Ser Asn Ser	
	325 330 335	
65	TGC CAA GAC TCC ACG GAC ACC GAG AGC AAC AAC GAG GAG CAG CGC AGC	1056
	Cys Gln Asp Ser Thr Asp Thr Glu Ser Asn Asn Glu Glu Gln Arg Ser	
	340 345 350	
70	GGT CTT ATC TAC CTG ACC AAC CAC ATC GCC CGA CGC GCG CAA CGC GTG	1104

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	Gly	Leu	Ile	Tyr	Leu	Thr	Asn	His	Ile	Ala	Arg	Arg	Ala	Gln	Arg	Val	
	355							360					365				
5	TCG	CTC	AAG	GAG	GAG	CAC	CGC	GCC	TAC	GAC	CTG	CTG	CGC	GCC	GCC	TCC	1152
	Ser	Leu	Lys	Glu	Glu	His	Arg	Ala	Tyr	Asp	Leu	Leu	Arg	Ala	Ala	Ser	
	370						375				380						
10	GAG	AAC	TCG	CAG	GAC	GCG	CTC	CGC	GTG	GTC	AGC	ACC	AGC	GGG	GAG	CAG	1200
	Glu	Asn	Ser	Gln	Asp	Ala	Leu	Arg	Val	Val	Ser	Thr	Ser	Gly	Glu	Gln	
	385					390				395					400		
15	ATG	AAG	GTG	TAC	AAG	TGC	GAA	CAC	TGC	CGG	GTG	CTC	TTC	CTG	GAT	CAC	1248
	Met	Lys	Val	Tyr	Lys	Cys	Glu	His	Cys	Arg	Val	Leu	Phe	Leu	Asp	His	
					405				410						415		
	GTC	ATG	TAC	ACC	ATC	CAC	ATG	GGC	TGC	CAC	GGC	TTC	CGT	GAT	CCT	TTT	1296
	Val	Met	Tyr	Thr	Ile	His	Met	Gly	Cys	His	Gly	Phe	Arg	Asp	Pro	Phe	
				420				425				430					
20	GAG	TGC	AAC	ATG	TGC	GGC	TAC	CAC	AGC	CAG	GAC	CGG	TAC	GAG	TTC	TCG	1344
	Glu	Cys	Asn	Met	Cys	Gly	Tyr	His	Ser	Gln	Asp	Arg	Tyr	Glu	Phe	Ser	
		435				440					445						
25	TCG	CAC	ATA	ACG	CGA	GGG	GAG	CAC	CGC	TTC	CAC	ATG	AGC	TAA			1386
	Ser	His	Ile	Thr	Arg	Gly	Glu	His	Arg	Phe	His	Met	Ser				
		450				455				460							

(2) INFORMATION FOR SEQ ID NO:17:

- 30 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1296 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
- (A) NAME/KEY: CDS
 - (B) LOCATION: 1..1296
- 40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

45	ATG	GAT	GTC	GAT	GAG	GGT	CAA	GAC	ATG	TCC	CAA	GTT	TCA	GGA	AAG	GAG	48
	Met	Asp	Val	Asp	Glu	Gly	Gln	Asp	Met	Ser	Gln	Val	Ser	Gly	Lys	Glu	
	1				5				10					15			
50	AGC	CCC	CCA	GTC	AGT	GAC	ACT	CCA	GAT	GAA	GGG	GAT	GAG	CCC	ATG	CCT	96
	Ser	Pro	Pro	Val	Ser	Asp	Thr	Pro	Asp	Glu	Gly	Asp	Glu	Pro	Met	Pro	
				20				25					30				
55	GTC	CCT	GAG	GAC	CTG	TCC	ACT	ACC	TCT	GGA	GCA	CAG	CAG	AAC	TCC	AAG	144
	Val	Pro	Glu	Asp	Leu	Ser	Thr	Thr	Ser	Gly	Ala	Gln	Gln	Asn	Ser	Lys	
			35				40					45					
	AGT	GAT	CGA	GGC	ATG	GCC	AGT	AAT	GTT	AAA	GTA	GAG	ACT	CAG	AGT	GAT	192
	Ser	Asp	Arg	Gly	Met	Ala	Ser	Asn	Val	Lys	Val	Glu	Thr	Gln	Ser	Asp	
		50				55					60						

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	GAA GAG AAT GGG CGT GCC TGT GAA ATG AAT GGG GAA GAA TGT GCA GAG	240
	Glu Glu Asn Gly Arg Ala Cys Glu Met Asn Gly Glu Glu Cys Ala Glu	
	65 70 75 80	
5	GAT TTA CGA ATG CTT GAT GCC TCG GGA GAG AAA ATG AAT GGC TCC CAC	288
	Asp Leu Arg Met Leu Asp Ala Ser Gly Glu Lys Met Asn Gly Ser His	
	85 90 95	
10	AGG GAC CAA GGC AGC TCG GCT TTG TCA GGA GTT GGA GGC ATT CGA CTT	336
	Arg Asp Gln Gly Ser Ser Ala Leu Ser Gly Val Gly Gly Ile Arg Leu	
	100 105 110	
15	CCT AAC GGA AAA CTA AAG TGT GAT ATC TGT GGG ATC GTT TGC ATC GGG	384
	Pro Asn Gly Lys Leu Lys Cys Asp Ile Cys Gly Ile Val Cys Ile Gly	
	115 120 125	
20	CCC AAT GTG CTC ATG GTT CAC AAA AGA AGT CAT ACT GGT GAA CGG CCT	432
	Pro Asn Val Leu Met Val His Lys Arg Ser His Thr Gly Glu Arg Pro	
	130 135 140	
25	TTC CAG TGC AAC CAG TCT GGG GCC TCC TTT ACC CAG AAA GGC AAC CTC	480
	Phe Gln Cys Asn Gln Ser Gly Ala Ser Phe Thr Gln Lys Gly Asn Leu	
	145 150 155 160	
30	CTG CGG CAC ATC AAG CTG CAC TCG GGT GAG AAG CCC TTC AAA TGC CAT	528
	Leu Arg His Ile Lys Leu His Ser Gly Glu Lys Pro Phe Lys Cys His	
	165 170 175	
35	CTT TGC AAC TAT GCC TGC CGC CGG AGG GAC GCC CTC ACC GGC CAC CTG	576
	Leu Cys Asn Tyr Ala Cys Arg Arg Arg Asp Ala Leu Thr Gly His Leu	
	180 185 190	
40	AGG ACG CAC TCC GGA GAC AAG TGC CTG TCA GAC ATG CCC TAT GAC AGT	624
	Arg Thr His Ser Gly Asp Lys Cys Leu Ser Asp Met Pro Tyr Asp Ser	
	195 200 205	
45	GCC AAC TAT GAG AAG GAG GAT ATG ATG ACA TCC CAC GTG ATG GAC CAG	672
	Ala Asn Tyr Glu Lys Glu Asp Met Met Thr Ser His Val Met Asp Gln	
	210 215 220	
50	GCC ATC AAC AAT GCC ATC AAC TAC CTG GGG GCT GAG TCC CTG CGC CCA	720
	Ala Ile Asn Asn Ala Ile Asn Tyr Leu Gly Ala Glu Ser Leu Arg Pro	
	225 230 235 240	
55	TTG GTG CAG ACA CCC CCC GGT AGC TCC GAG GTG GTG CCA GTC ATC AGC	768
	Leu Val Gln Thr Pro Pro Gly Ser Ser Glu Val Val Pro Val Ile Ser	
	245 250 255	
60	TCC ATG TAC CAG CTG CAC AAG CCC CCC TCA GAT GGC CCC CCA CGG TCC	816
	Ser Met Tyr Gln Leu His Lys Pro Pro Ser Asp Gly Pro Pro Arg Ser	
	260 265 270	
65	AAC CAT TCA GCA CAG GAC GCC GTG GAT AAC TTG CTG CTG CTG TCC AAG	864
	Asn His Ser Ala Gln Asp Ala Val Asp Asn Leu Leu Leu Ser Lys	
	275 280 285	
70	GCC AAG TCT GTG TCA TCG GAG CGA GAG GCC TCC CCG AGC AAC AGC TGC	912

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	Ala Lys Ser Val Ser Ser Glu Arg Glu Ala Ser Pro Ser Asn Ser Cys	
	290 295 300	
5	CAA GAC TCC ACA GAT ACA GAG AGC AAC GCG GAG GAA CAG CGC AGC GGC Gln Asp Ser Thr Asp Thr Glu Ser Asn Ala Glu Glu Gln Arg Ser Gly	960
	305 310 315 320	
10	CTT ATC TAC CTA ACC AAC CAC ATC AAC CCG CAT GCA CGC AAT GGG CTG Leu Ile Tyr Leu Thr Asn His Ile Asn Pro His Ala Arg Asn Gly Leu	1008
	325 330 335	
15	GCT CTC AAG GAG GAG CAG CGC GCC TAC GAG GTG CTG AGG GCG GCC TCA Ala Leu Lys Glu Glu Gln Arg Ala Tyr Glu Val Leu Arg Ala Ala Ser	1056
	340 345 350	
	GAG AAC TCG CAG GAT GCC TTC CGT GTG GTC AGC ACG AGT GGC GAG CAG Glu Asn Ser Gln Asp Ala Phe Arg Val Val Ser Thr Ser Gly Glu Gln	1104
	355 360 365	
20	CTG AAG GTG TAC AAG TGC GAA CAC TGC CGC GTG CTC TTC CTG GAT CAC Leu Lys Val Tyr Lys Cys Glu His Cys Arg Val Leu Phe Leu Asp His	1152
	370 375 380	
25	GTC ATG TAT ACC ATT CAC ATG GGC TGC CAT GGC TGC CAT GGC TTT CGG Val Met Tyr Thr Ile His Met Gly Cys His Gly Cys His Gly Phe Arg	1200
	385 390 395 400	
30	GAT CCC TTT GAG TGT AAC ATG TGT GGT TAT CAC AGC CAG GAC AGG TAC Asp Pro Phe Glu Cys Asn Met Cys Gly Tyr His Ser Gln Asp Arg Tyr	1248
	405 410 415	
35	GAG TTC TCA TCC CAT ATC ACG CGG GGG GAG CAT CGT TAC CAC CTG AGC Glu Phe Ser Ser His Ile Thr Arg Gly Glu His Arg Tyr His Leu Ser	1296
	420 425 430	
	(2) INFORMATION FOR SEQ ID NO:18:	
	(i) SEQUENCE CHARACTERISTICS:	
40	(A) LENGTH: 2049 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: double	
	(D) TOPOLOGY: linear	
45	(ii) MOLECULE TYPE: cDNA	
	(ix) FEATURE:	
	(A) NAME/KEY: CDS	
	(B) LOCATION: 223..1776	
50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:	
	AATTCGTTCT ACCTTCTCTG AACCCCAAGTG GTGTGTCAAG GCCGGACTGG GAGCTTGGGG	60
55	GAAGAGGAAG AGGAAGAGGA ATCTGCGGCT CATCCAGGGA TCAGGGTCCT TCCCAAGTGG	120
	CCACTCAGAG GGGACTCAGA GCAAGTCTAG ATTTGTGTGG CAGAGAGAGA CAGCTCTCGT	180
	TTGGCCTTGG GGAGGCACAA GTCTGTTGAT AACCTGAAGA CA ATG GAT GTC GAT	234

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Met Asp Val Asp

1

5	GAG GGT CAA GAC ATG TCC CAA GTT TCA GGA AAG GAG AGC CCC CCA GTC	282
	Glu Gly Gln Asp Met Ser Gln Val Ser Gly Lys Glu Ser Pro Pro Val	
	5 10 15 20	
10	AGT GAC ACT CCA GAT GAA GGG GAT GAG CCC ATG CCT GTC CCT GAG GAC	330
	Ser Asp Thr Pro Asp Glu Gly Asp Glu Pro Met Pro Val Pro Glu Asp	
	25 30 35	
15	CTG TCC ACT ACC TCT GGA GCA CAG CAG AAC TCC AAG AGT GAT CGA GGC	378
	Leu Ser Thr Thr Ser Gly Ala Gln Gln Asn Ser Lys Ser Asp Arg Gly	
	40 45 50	
20	ATG GCC AGT AAT GTT AAA GTA GAG ACT CAG AGT GAT GAA GAG AAT GGG	426
	Met Ala Ser Asn Val Lys Val Glu Thr Gln Ser Asp Glu Glu Asn Gly	
	55 60 65	
25	CGT GCC TGT GAA ATG AAT GGG GAA GAA TGT GCA GAG GAT TTA CGA ATG	474
	Arg Ala Cys Glu Met Asn Gly Glu Glu Cys Ala Glu Asp Leu Arg Met	
	70 75 80	
30	CTT GAT GCC TCG GGA GAG AAA ATG AAT GGC TCC CAC AGG GAC CAA GGC	522
	Leu Asp Ala Ser Gly Glu Lys Met Asn Gly Ser His Arg Asp Gln Gly	
	85 90 95 100	
35	AGC TCG GCT TTG TCA GGA GTT GGA GGC ATT CGA CTT CCT AAC GGA AAA	570
	Ser Ser Ala Leu Ser Gly Val Gly Gly Ile Arg Leu Pro Asn Gly Lys	
	105 110 115	
40	CTA AAG TGT GAT ATC TGT GGG ATC GTT TGC ATC GGG CCC AAT GTG CTC	618
	Leu Lys Cys Asp Ile Cys Gly Ile Val Cys Ile Gly Pro Asn Val Leu	
	120 125 130	
45	ATG GTT CAC AAA AGA AGT CAT ACT GGT GAA CGG CCT TTC CAG TGC AAC	666
	Met Val His Lys Arg Ser His Thr Gly Glu Arg Pro Phe Gln Cys Asn	
	135 140 145	
50	CAG TCT GGG GCC TCC TTT ACC CAG AAA GGC AAC CTC CTG CGG CAC ATC	714
	Gln Ser Gly Ala Ser Phe Thr Gln Lys Gly Asn Leu Leu Arg His Ile	
	150 155 160	
55	AAG CTG CAC TCG GGT GAG AAG CCC TTC AAA TGC CAT CTT TGC AAC TAT	762
	Lys Leu His Ser Gly Glu Lys Pro Phe Lys Cys His Leu Cys Asn Tyr	
	165 170 175 180	
60	GCC TGC CGC CGG AGG GAC GCC CTC ACC GGC CAC CTG AGG ACG CAC TCC	810
	Ala Cys Arg Arg Arg Asp Ala Leu Thr Gly His Leu Arg Thr His Ser	
	185 190 195	
65	GTT GGT AAG CCT CAC AAA TGT GGA TAT TGT GGC CGG AGC TAT AAA CAG	858
	Val Gly Lys Pro His Lys Cys Gly Tyr Cys Gly Arg Ser Tyr Lys Gln	
	200 205 210	
70	CGA AGC TCT TTA GAG GAG CAT AAA GAG CGA TGC CAC AAC TAC TTG GAA	906
	Arg Ser Ser Leu Glu Glu His Lys Glu Arg Cys His Asn Tyr Leu Glu	
	215 220 225	

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	AGC ATG GGC CTT CCG GGC GTG TGC CCA GTC ATT AAG GAA GAA ACT AAC	954
	Ser Met Gly Leu Pro Gly Val Cys Pro Val Ile Lys Glu Glu Thr Asn	
	230 235 240	
5	CAC AAC GAG ATG GCA GAA GAC CTG TGC AAG ATA GGA GCA GAG AGG TCC	1002
	His Asn Glu Met Ala Glu Asp Leu Cys Lys Ile Gly Ala Glu Arg Ser	
	245 250 255 260	
10	CTT GTC CTG GAC AGG CTG GCA AGC AAT GTC GCC AAA CGT AAG AGC TCT	1050
	Leu Val Leu Asp Arg Leu Ala Ser Asn Val Ala Lys Arg Lys Ser Ser	
	265 270 275	
15	ATG CCT CAG AAA TTT CTT GGA GAC AAG TGC CTG TCA GAC ATG CCC TAT	1098
	Met Pro Gln Lys Phe Leu Gly Asp Lys Cys Leu Ser Asp Met Pro Tyr	
	280 285 290	
20	GAC AGT GCC AAC TAT GAG AAG GAG GAT ATG ATG ACA TCC CAC GTG ATG	1146
	Asp Ser Ala Asn Tyr Glu Lys Glu Asp Met Met Thr Ser His Val Met	
	295 300 305	
25	GAC CAG GCC ATC AAC AAT GCC ATC AAC TAC CTG GGG GCT GAG TCC CTG	1194
	Asp Gln Ala Ile Asn Asn Ala Ile Asn Tyr Leu Gly Ala Glu Ser Leu	
	310 315 320	
30	CGC CCA TTG GTG CAG ACA CCC CCC GGT AGC TCC GAG GTG GTG CCA GTC	1242
	Afg Pro Leu Val Gln Thr Pro Pro Gly Ser Ser Glu Val Val Pro Val	
	325 330 335 340	
35	ATC AGC TCC ATG TAC CAG CTG CAC AAG CCC CCC TCA GAT GGC CCC CCA	1290
	Ile Ser Ser Met Tyr Gln Leu His Lys Pro Pro Ser Asp Gly Pro Pro	
	345 350 355	
40	CGG TCC AAC CAT TCA GCA CAG GAC GCC GTG GAT AAC TTG CTG CTG CTG	1338
	Arg Ser Asn His Ser Ala Gln Asp Ala Val Asp Asn Leu Leu Leu Leu	
	360 365 370	
45	TCC AAG GCC AAG TCT GTG TCA TCG GAG CGA GAG GCC TCC CCG AGC AAC	1386
	Ser Lys Ala Lys Ser Val Ser Ser Glu Arg Glu Ala Ser Pro Ser Asn	
	375 380 385	
50	AGC TGC CAA GAC TCC ACA GAT ACA GAG AGC AAC GCG GAG GAA CAG CGC	1434
	Ser Cys Gln Asp Ser Thr Asp Thr Glu Ser Asn Ala Glu Glu Gln Arg	
	390 395 400	
55	AGC GGC CTT ATC TAC CTA ACC AAC CAC ATC AAC CCG CAT GCA CGC AAT	1482
	Ser Gly Leu Ile Tyr Leu Thr Asn His Ile Asn Pro His Ala Arg Asn	
	405 410 415 420	
60	GGG CTG GCT CTC AAG GAG GAG CAG CGC GCC TAC GAG GTG CTG AGG GCG	1530
	Gly Leu Ala Leu Lys Glu Glu Gln Arg Ala Tyr Glu Val Leu Arg Ala	
	425 430 435	
65	GCC TCA GAG AAC TCG CAG GAT GCC TTC CGT GTG GTC AGC ACG AGT GGC	1578
	Ala Ser Glu Asn Ser Gln Asp Ala Phe Arg Val Val Ser Thr Ser Gly	
	440 445 450	
70	GAG CAG CTG AAG GTG TAC AAG TGC GAA CAC TGC CGC GTG CTC TTC CTG	1626

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Glu Gln Leu Lys Val Tyr Lys Cys Glu His Cys Arg Val Leu Phe Leu
 455 460 465

5 GAT CAC GTC ATG TAT ACC ATT CAC ATG GGC TGC CAT GGC TGC CAT GGC 1674
 Asp His Val Met Tyr Thr Ile His Met Gly Cys His Gly Cys His Gly
 470 475 480

10 TTT CGG GAT CCC TTT GAG TGT AAC ATG TGT GGT TAT CAC AGC CAG GAC 1722
 Phe Arg Asp Pro Phe Glu Cys Asn Met Cys Gly Tyr His Ser Gln Asp
 485 490 495 500

AGG TAC GAG TTC TCA TCC CAT ATC ACG CGG GGG GAG CAT CGT TAC CAC 1770
 Arg Tyr Glu Phe Ser Ser His Ile Thr Arg Gly Glu His Arg Tyr His
 505 510 515

15 CTG AGC TAAACCCAGC CAGGCCCCAC TGAAGCACAA AGATAGCTGG TTATGCCTCC 1826
 Leu Ser

20 TTCCCGGCAG CTGGACCCAC AGCGGACAAT GTGGGAGTGG ATTTGCAGGC AGCATTGTGT 1886
 CTTTTATGTT GGTGTGTTGG CGTTTCATTT GCGTTGGAAG ATAAGTTTTT AATGTTAGTG 1946

25 ACAGGATTGC ATTGCATCAG CAACATTAC AACATCCATC CTTCTAGCCA GTTTTGTTC 2006
 CTGGTAGCTG AGGTTTCCCG GATATGTGGC TTCCTAACAC TCT 2049

(2) INFORMATION FOR SEQ ID NO:19:

- 30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1170 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 40 (B) LOCATION: 1..1170
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

45 ATG GAT GTC GAT GAG GGT CAA GAC ATG TCC CAA GTT TCA GGA AAG GAG 48
 Met Asp Val Asp Glu Gly Gln Asp Met Ser Gln Val Ser Gly Lys Glu
 1 5 10 15

AGC CCC CCA GTC AGT GAC ACT CCA GAT GAA GGG GAT GAG CCC ATG CCT 96
 Ser Pro Pro Val Ser Asp Thr Pro Asp Glu Gly Asp Glu Pro Met Pro
 50 20 25 30

GTC CCT GAG GAC CTG TCC ACT ACC TCT GGA GCA CAG CAG AAC TCC AAG 144
 Val Pro Glu Asp Leu Ser Thr Thr Ser Gly Ala Gln Gln Asn Ser Lys
 35 40 45

55 AGT GAT CGA GGC ATG GGT GAA CGG CCT TTC CAG TGC AAC CAG TCT GGG 192
 Ser Asp Arg Gly Met Gly Glu Arg Pro Phe Gln Cys Asn Gln Ser Gly
 50 55 60

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5	GCC TCC TTT ACC CAG AAA GGC AAC CTC CTG CGG CAC ATC AAG CTG CAC	240
	Ala Ser Phe Thr Gln Lys Gly Asn Leu Leu Arg His Ile Lys Leu His	
	65 70 75 80	
10	TCG GGT GAG AAG CCC TTC AAA TGC CAT CTT TGC AAC TAT GCC TGC CGC	288
	Ser Gly Glu Lys Pro Phe Lys Cys His Leu Cys Asn Tyr Ala Cys Arg	
	85 90 95	
15	CGG AGG GAC GCC CTC ACC GGC CAC CTG AGG ACG CAC TCC GTC ATT AAG	336
	Arg Arg Asp Ala Leu Thr Gly His Leu Arg Thr His Ser Val Ile Lys	
	100 105 110	
20	GAA GAA ACT AAC CAC AAC GAG ATG GCA GAA GAC CTG TGC AAG ATA GGA	384
	Glu Glu Thr Asn His Asn Glu Met Ala Glu Asp Leu Cys Lys Ile Gly	
	115 120 125	
25	GCA GAG AGG TCC CTT GTC CTG GAC AGG CTG GCA AGC AAT GTC GCC AAA	432
	Ala Glu Arg Ser Leu Val Leu Asp Arg Leu Ala Ser Asn Val Ala Lys	
	130 135 140	
30	CGT AAG AGC TCT ATG CCT CAG AAA TTT CTT GGA GAC AAG TGC CTG TCA	480
	Arg Lys Ser Ser Met Pro Gln Lys Phe Leu Gly Asp Lys Cys Leu Ser	
	145 150 155 160	
35	GAC ATG CCC TAT GAC AGT GCC AAC TAT GAG AAG GAG GAT ATG ATG ACA	528
	Asp Met Pro Tyr Asp Ser Ala Asn Tyr Glu Lys Glu Asp Met Met Thr	
	165 170 175	
40	TCC CAC GTG ATG GAC CAG GCC ATC AAC AAT GCC ATC AAC TAC CTG GGG	576
	Ser His Val Met Asp Gln Ala Ile Asn Asn Ala Ile Asn Tyr Leu Gly	
	180 185 190	
45	GCT GAG TCC CTG CGC CCA TTG GTG CAG ACA CCC CCC GGT AGC TCC GAG	624
	Ala Glu Ser Leu Arg Pro Leu Val Gln Thr Pro Pro Gly Ser Ser Glu	
	195 200 205	
50	GTG GTG CCA GTC ATC AGC TCC ATG TAC CAG CTG CAC AAG CCC CCC TCA	672
	Val Val Pro Val Ile Ser Ser Met Tyr Gln Leu His Lys Pro Pro Ser	
	210 215 220	
55	GAT GGC CCC CCA CGG TCC AAC CAT TCA GCA CAG GAC GCC GTG GAT AAC	720
	Asp Gly Pro Pro Arg Ser Asn His Ser Ala Gln Asp Ala Val Asp Asn	
	225 230 235 240	
60	TTG CTG CTG CTG TCC AAG GCC AAG TCT GTG TCA TCG GAG CGA GAG GCC	768
	Leu Leu Leu Leu Ser Lys Ala Lys Ser Val Ser Ser Glu Arg Glu Ala	
	245 250 255	
65	TCC CCG AGC AAC AGC TGC CAA GAC TCC ACA GAT ACA GAG AGC AAC GCG	816
	Ser Pro Ser Asn Ser Cys Gln Asp Ser Thr Asp Thr Glu Ser Asn Ala	
	260 265 270	
70	GAG GAA CAG CGC AGC GGC CTT ATC TAC CTA ACC AAC CAC ATC AAC CCG	864
	Glu Glu Gln Arg Ser Gly Leu Ile Tyr Leu Thr Asn His Ile Asn Pro	
	275 280 285	
75	CAT GCA CGC AAT GGG CTG GCT CTC AAG GAG GAG CAG CGC GCC TAC GAG	912

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His Ala Arg Asn Gly Leu Ala Leu Lys Glu Glu Gln Arg Ala Tyr Glu
 290 295 300

5 GTG CTG AGG GCG GCC TCA GAG AAC TCG CAG GAT GCC TTC CGT GTG GTC 960
 Val Leu Arg Ala Ala Ser Glu Asn Ser Gln Asp Ala Phe Arg Val Val
 305 310 315 320

10 AGC ACG AGT GGC GAG CAG CTG AAG GTG TAC AAG TGC GAA CAC TGC CGC 1008
 Ser Thr Ser Gly Glu Gln Leu Lys Val Tyr Lys Cys Glu His Cys Arg
 325 330 335

15 GTG CTC TTC CTG GAT CAC GTC ATG TAT ACC ATT CAC ATG GGC TGC CAT 1056
 Val Leu Phe Leu Asp His Val Met Tyr Thr Ile His Met Gly Cys His
 340 345 350

GGC TGC CAT GGC TTT CGG GAT CCC TTT GAG TGT AAC ATG TGT GGT TAT 1104
 Gly Cys His Gly Phe Arg Asp Pro Phe Glu Cys Asn Met Cys Gly Tyr
 355 360 365

20 CAC AGC CAG GAC AGG TAC GAG TTC TCA TCC CAT ATC ACG CGG GGG GAG 1152
 His Ser Gln Asp Arg Tyr Glu Phe Ser Ser His Ile Thr Arg Gly Glu
 370 375 380

25 CAT CGT TAC CAC CTG AGC 1170
 His Arg Tyr His Leu Ser
 385 390

(2) INFORMATION FOR SEQ ID NO:20:

- 30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1128 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 40 (B) LOCATION: 1..1128
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

45 ATG GAT GTC GAT GAG GGT CAA GAC ATG TCC CAA GTT TCA GGA AAG GAG 48
 Met Asp Val Asp Glu Gly Gln Asp Met Ser Gln Val Ser Gly Lys Glu
 1 5 10 15

AGC CCC CCA GTC AGT GAC ACT CCA GAT GAA GGG GAT GAG CCC ATG CCT 96
 Ser Pro Pro Val Ser Asp Thr Pro Asp Glu Gly Asp Glu Pro Met Pro
 50 20 25 30

GTC CCT GAG GAC CTG TCC ACT ACC TCT GGA GCA CAG CAG AAC TCC AAG 144
 Val Pro Glu Asp Leu Ser Thr Ser Gly Ala Gln Gln Asn Ser Lys
 35 40 45

55 AGT GAT CGA GGC ATG GCC AGT AAT GTT AAA GTA GAG ACT CAG AGT GAT 192
 Ser Asp Arg Gly Met Ala Ser Asn Val Lys Val Glu Thr Gln Ser Asp
 50 55 60

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5	GAA GAG AAT GGG CGT GCC TGT GAA ATG AAT GGG GAA GAA TGT GCA GAG	240
	Glu Glu Asn Gly Arg Ala Cys Glu Met Asn Gly Glu Glu Cys Ala Glu	
	65 70 75 80	
10	GAT TTA CGA ATG CTT GAT GCC TCG GGA GAG AAA ATG AAT GGC TCC CAC	288
	Asp Leu Arg Met Leu Asp Ala Ser Gly Glu Lys Met Asn Gly Ser His	
	85 90 95	
15	AGG GAC CAA GGC AGC TCG GCT TTG TCA GGA GTT GGA GGC ATT CGA CTT	336
	Arg Asp Gln Gly Ser Ser Ala Leu Ser Gly Val Gly Gly Ile Arg Leu	
	100 105 110	
20	CCT AAC GGA AAA CTA AAG TGT GAT ATC TGT GGG ATC GTT TGC ATC GGG	384
	Pro Asn Gly Lys Leu Lys Cys Asp Ile Cys Gly Ile Val Cys Ile Gly	
	115 120 125	
25	CCC AAT GTG CTC ATG GTT CAC AAA AGA AGT CAT ACT GGA GAC AAG TGC	432
	Pro Asn Val Leu Met Val His Lys Arg Ser His Thr Gly Asp Lys Cys	
	130 135 140	
30	CTG TCA GAC ATG CCC TAT GAC AGT GCC AAC TAT GAG AAG GAG GAT ATG	480
	Leu Ser Asp Met Pro Tyr Asp Ser Ala Asn Tyr Glu Lys Glu Asp Met	
	145 150 155 160	
35	ATG ACA TCC CAC GTG ATG GAC CAG GCC ATC AAC AAT GCC ATC AAC TAC	528
	Mét Thr Ser His Val Met Asp Gln Ala Ile Asn Asn Ala Ile Asn Tyr	
	165 170 175	
40	CTG GGG GCT GAG TCC CTG CGC CCA TTG GTG CAG ACA CCC CCC GGT AGC	576
	Leu Gly Ala Glu Ser Leu Arg Pro Leu Val Gln Thr Pro Pro Gly Ser	
	180 185 190	
45	TCC GAG GTG GTG CCA GTC ATC AGC TCC ATG TAC CAG CTG CAC AAG CCC	624
	Ser Glu Val Val Pro Val Ile Ser Ser Met Tyr Gln Leu His Lys Pro	
	195 200 205	
50	CCC TCA GAT GGC CCC CCA CGG TCC AAC CAT TCA GCA CAG GAC GCC GTG	672
	Pro Ser Asp Gly Pro Pro Arg Ser Asn His Ser Ala Gln Asp Ala Val	
	210 215 220	
55	GAT AAC TTG CTG CTG CTG TCC AAG GCC AAG TCT GTG TCA TCG GAG CGA	720
	Asp Asn Leu Leu Leu Leu Ser Lys Ala Lys Ser Val Ser Ser Glu Arg	
	225 230 235 240	
60	GAG GCC TCC CCG AGC AAC AGC TGC CAA GAC TCC ACA GAT ACA GAG AGC	768
	Glu Ala Ser Pro Ser Asn Ser Cys Gln Asp Ser Thr Asp Thr Glu Ser	
	245 250 255	
65	AAC GCG GAG GAA CAG CGC AGC GGC CTT ATC TAC CTA ACC AAC CAC ATC	816
	Asn Ala Glu Glu Gln Arg Ser Gly Leu Ile Tyr Leu Thr Asn His Ile	
	260 265 270	
70	AAC CCG CAT GCA CGC AAT GGG CTG GCT CTC AAG GAG GAG CAG CGC GCC	864
	Asn Pro His Ala Arg Asn Gly Leu Ala Leu Lys Glu Glu Gln Arg Ala	
	275 280 285	
75	TAC GAG GTG CTG AGG GCG GCC TCA GAG AAC TCG CAG GAT GCC TTC CGT	912

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	Tyr	Glu	Val	Leu	Arg	Ala	Ala	Ser	Glu	Asn	Ser	Gln	Asp	Ala	Phe	Arg	
	290					295					300						
5	GTG	GTC	AGC	ACG	AGT	GGC	GAG	CAG	CTG	AAG	GTG	TAC	AAG	TGC	GAA	CAC	960
	Val	Val	Ser	Thr	Ser	Gly	Glu	Gln	Leu	Lys	Val	Tyr	Lys	Cys	Glu	His	
	305					310					315					320	
10	TGC	CGC	GTG	CTC	TTC	CTG	GAT	CAC	GTC	ATG	TAT	ACC	ATT	CAC	ATG	GGC	1008
	Cys	Arg	Val	Leu	Phe	Leu	Asp	His	Val	Met	Tyr	Thr	Ile	His	Met	Gly	
					325					330					335		
15	TGC	CAT	GGC	TGC	CAT	GGC	TTT	CGG	GAT	CCC	TTT	GAG	TGT	AAC	ATG	TGT	1056
	Cys	His	Gly	Cys	His	Gly	Phe	Arg	Asp	Pro	Phe	Glu	Cys	Asn	Met	Cys	
				340					345					350			
20	GGT	TAT	CAC	AGC	CAG	GAC	AGG	TAC	GAG	TTC	TCA	TCC	CAT	ATC	ACG	CGG	1104
	Gly	Tyr	His	Ser	Gln	Asp	Arg	Tyr	Glu	Phe	Ser	Ser	His	Ile	Thr	Arg	
			355					360						365			
25	GGG	GAG	CAT	CGT	TAC	CAC	CTG	AGC									1128
	Gly	Glu	His	Arg	Tyr	His	Leu	Ser									
		370					375										
30	(2) INFORMATION FOR SEQ ID NO:21:																
	(i) SEQUENCE CHARACTERISTICS:																
	(A) LENGTH: 1004 base pairs																
	(B) TYPE: nucleic acid																
	(C) STRANDEDNESS: single																
	(D) TOPOLOGY: linear																
35	(ii) MOLECULE TYPE: cDNA																
	(ix) FEATURE:																
	(A) NAME/KEY: CDS																
	(B) LOCATION: 1..1002																
40	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:																
	GGA	GAA	CGG	CCC	TTC	CAG	TGC	AAT	CAG	TGC	GGG	GCC	TCA	TTC	ACC	CAG	48
	Gly	Glu	Arg	Pro	Phe	Gln	Cys	Asn	Gln	Cys	Gly	Ala	Ser	Phe	Thr	Gln	
	1				5					10					15		
45	AAG	GGC	AAC	CTG	CTC	CGG	CAC	ATC	AAG	CTG	CAT	TCC	GGG	GAG	AAG	CCC	96
	Lys	Gly	Asn	Leu	Leu	Arg	His	Ile	Lys	Leu	His	Ser	Gly	Glu	Lys	Pro	
				20					25					30			
50	TTC	AAA	TGC	CAC	CTC	TGC	AAC	TAC	GCC	TGC	CGC	CGG	AGG	GAC	GCC	CTC	144
	Phe	Lys	Cys	His	Leu	Cys	Asn	Tyr	Ala	Cys	Arg	Arg	Arg	Asp	Ala	Leu	
			35					40					45				
55	ACT	GGC	CAC	CTG	AGG	ACG	CAC	TCC	GTC	ATT	AAA	GAA	GAA	ACT	AAG	CAC	192
	Thr	Gly	His	Leu	Arg	Thr	His	Ser	Val	Ile	Lys	Glu	Glu	Thr	Lys	His	
		50					55					60					
60	AGT	GAA	ATG	GCA	GAA	GAC	CTG	TGC	AAG	ATA	GGA	TCA	GAG	AGA	TCT	CTC	240
	Ser	Glu	Met	Ala	Glu	Asp	Leu	Cys	Lys	Ile	Gly	Ser	Glu	Arg	Ser	Leu	
		65					70					75				80	

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	GTG CTG GAC AGA CTA GCA AGT AAT GTC GCC AAA CGT AAG AGC TCT ATG	288
	Val Leu Asp Arg Leu Ala Ser Asn Val Ala Lys Arg Lys Ser Ser Met	
	85 90 95	
5	CCT CAG AAA TTT CTT GGG GAC AAG GGC CTG TCC GAC ACG CCC TAC GAC	336
	Pro Gln Lys Phe Leu Gly Asp Lys Gly Leu Ser Asp Thr Pro Tyr Asp	
	100 105 110	
10	AGT GCC ACG TAC GAG AAG GAG AAC GAA ATG ATG AAG TCC CAC GTG ATG	384
	Ser Ala Thr Tyr Glu Lys Glu Asn Glu Met Met Lys Ser His Val Met	
	115 120 125	
15	GAC CAA GCC ATC AAC AAC GCC ATC AAC TAC CTG GGG GCC GAG TCC CTG	432
	Asp Gln Ala Ile Asn Asn Ala Ile Asn Tyr Leu Gly Ala Glu Ser Leu	
	130 135 140	
20	CGC CCG CTG GTG CAG ACG CCC CCG GGC GGT TCC GAG GTG GTC CCG GTC	480
	Arg Pro Leu Val Gln Thr Pro Pro Gly Gly Ser Glu Val Val Pro Val	
	145 150 155 160	
	ATC AGC CCG ATG TAC CAG CTG CAC AGG CGC TCG GAG GGC ACC CCG CGC	528
	Ile Ser Pro Met Tyr Gln Leu His Arg Arg Ser Glu Gly Thr Pro Arg	
	165 170 175	
25	TCC AAC CAC TCG GCC CAG GAC AGC GCC GTG GAG TAC CTG CTG CTG CTC	576
	Sér Asn His Ser Ala Gln Asp Ser Ala Val Glu Tyr Leu Leu Leu Leu	
	180 185 190	
30	TCC AAG GCC AAG TTG GTG CCC TCG GAG CGC GAG GCG TCC CCG AGC AAC	624
	Ser Lys Ala Lys Leu Val Pro Ser Glu Arg Glu Ala Ser Pro Ser Asn	
	195 200 205	
35	AGC TGC CAA GAC TCC ACG GAC ACC GAG AGC AAC AAC GAG GAG CAG CGC	672
	Ser Cys Gln Asp Ser Thr Asp Thr Glu Ser Asn Asn Glu Glu Gln Arg	
	210 215 220	
40	AGC GGT CTT ATC TAC CTG ACC AAC CAC ATC GCC CGA CGC GCG CAA CGC	720
	Ser Gly Leu Ile Tyr Leu Thr Asn His Ile Ala Arg Arg Ala Gln Arg	
	225 230 235 240	
	GTG TCG CTC AAG GAG GAG CAC CGC GCC TAC GAC CTG CTG CGC GCC GCC	768
	Val Ser Leu Lys Glu Glu His Arg Ala Tyr Asp Leu Leu Arg Ala Ala	
	245 250 255	
45	TCC GAG AAC TCG CAG GAC GCG CTC CGC GTG GTC AGC ACC AGC GGG GAG	816
	Ser Glu Asn Ser Gln Asp Ala Leu Arg Val Val Ser Thr Ser Gly Glu	
	260 265 270	
50	CAG ATG AAG GTG TAC AAG TGC GAA CAC TGC CGG GTG CTC TTC CTG GAT	864
	Gln Met Lys Val Tyr Lys Cys Glu His Cys Arg Val Leu Phe Leu Asp	
	275 280 285	
55	CAC GTC ATG TAC ACC ATC CAC ATG GGC TGC CAC GGC TTC CGT GAT CCT	912
	His Val Met Tyr Thr Ile His Met Gly Cys His Gly Phe Arg Asp Pro	
	290 295 300	
	TTT GAG TGC AAC ATG TGC GGC TAC CAC AGC CAG GAC CGG TAC GAG TTC	960

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Phe Glu Cys Asn Met Cys Gly Tyr His Ser Gln Asp Arg Tyr Glu Phe
 305 310 315 320

TCG TCG CAC ATA ACG CGA GGG GAG CAC CGC TTC CAC ATG AGC TA 1004
 5 Ser Ser His Ile Thr Arg Gly Glu His Arg Phe His Met Ser
 325 330

(2) INFORMATION FOR SEQ ID NO:22:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 470 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Xaa Xaa Ala Ser Asn Val Lys Val Glu Thr Gln Ser Asp Glu Glu Asn
 1 5 10 15
 25 Gly Arg Ala Cys Glu Met Asn Gly Glu Glu Cys Ala Glu Asp Leu Arg
 20 25 30
 Met Leu Asp Ala Ser Gly Glu Lys Met Asn Gly Ser His Arg Asp Gln
 35 40 45
 30 Gly Ser Ser Ala Leu Ser Gly Val Gly Gly Ile Arg Leu Pro Asn Gly
 50 55 60
 Lys Leu Lys Cys Asp Ile Cys Gly Ile Xaa Cys Ile Gly Pro Asn Val
 35 65 70 75 80
 Leu Met Val His Lys Arg Ser His Thr Gly Glu Arg Pro Phe Gln Cys
 85 90 95
 40 Asn Gln Cys Gly Ala Ser Phe Thr Gln Lys Gly Asn Leu Leu Arg His
 100 105 110
 Ile Lys Leu His Ser Gly Glu Lys Pro Phe Lys Cys His Leu Cys Asn
 115 120 125
 45 Tyr Ala Cys Arg Arg Arg Asp Ala Leu Thr Gly His Leu Arg Thr His
 130 135 140
 Ser Val Gly Lys Pro His Lys Cys Gly Tyr Cys Gly Arg Ser Tyr Lys
 50 145 150 155 160
 Gln Arg Xaa Ser Leu Glu Glu His Lys Glu Arg Cys His Asn Tyr Leu
 165 170 175
 55 Glu Ser Met Gly Leu Pro Gly Xaa Xaa Xaa Pro Val Ile Lys Glu Glu
 180 185 190
 Thr Xaa His Xaa Glu Met Ala Glu Asp Leu Cys Lys Ile Gly Xaa Glu

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	195		200		205
	Arg Ser Leu Val Leu Asp	Arg Leu Ala Ser Asn	Val Ala Lys Arg Lys		
	210	215	220		
5	Ser Ser Met Pro Gln Lys Phe Leu Gly Asp Lys Xaa Leu Ser Asp Xaa				
	225	230	235		240
	Pro Tyr Asp Ser Ala Xaa Tyr Glu Lys Glu Xaa Xaa Met Met Xaa Ser				
10		245	250		255
	His Val Met Asp Xaa Ala Ile Asn Asn Ala Ile Asn Tyr Leu Gly Ala				
		260	265		270
15	Glu Ser Leu Arg Pro Leu Val Gln Thr Pro Pro Gly Xaa Ser Glu Val				
		275	280		285
	Val Pro Val Ile Ser Pro Met Tyr Gln Leu His Xaa Xaa Xaa Ser Xaa				
20		290	295		300
	Gly Xaa Pro Arg Ser Asn His Ser Ala Gln Asp Xaa Ala Val Xaa Xaa				
	305	310	315		320
	Leu Leu Leu Leu Ser Lys Ala Lys Xaa Val Xaa Ser Glu Arg Glu Ala				
25		325	330		335
	Ser Pro Ser Asn Ser Cys Gln Asp Ser Thr Asp Thr Glu Ser Asn Xaa				
		340	345		350
30	Glu Glu Gln Arg Ser Gly Leu Ile Tyr Leu Thr Asn His Ile Xaa Xaa				
		355	360		365
	Xaa Ala Xaa Xaa Xaa Xaa Xaa Leu Lys Glu Glu Xaa Arg Ala Tyr Xaa				
35		370	375		380
	Xaa Leu Arg Ala Ala Ser Glu Asn Ser Gln Asp Ala Xaa Arg Val Val				
	385	390	395		400
	Ser Thr Ser Gly Glu Gln Xaa Lys Val Tyr Lys Cys Glu His Cys Arg				
40		405	410		415
	Val Leu Phe Leu Asp His Val Met Tyr Thr Ile His Met Xaa Xaa Xaa				
		420	425		430
45	Gly Cys His Gly Phe Arg Asp Pro Phe Glu Cys Asn Met Cys Gly Tyr				
		435	440		445
	His Ser Gln Asp Arg Tyr Glu Phe Ser Ser His Ile Thr Arg Gly Glu				
		450	455		460
50	His Arg Xaa His Xaa Ser				
	465	470			

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Claims:

1. A substantially pure preparation of an Aiolos polypeptide having the following properties.
 - 5 (a) it can form a dimer with an Aiolos or Ikaros polypeptide;
 - (b) it is expressed in committed lymphoid progenitors;
 - (c) it is expressed in committed T and B cells;
 - (d) it has a molecular weight of approximately 58 kD;
 - (e) it has at least one zinc finger domain;
 - 10 (f) it is not expressed in stem cells; and
 - (g) it is a transcriptional activator of a lymphoid gene.
2. A fragment of the protein of claim 1 at least 50 amino acids in length.
- 15 3. An anti Aiolos antibody.
4. A substantially pure nucleic acid comprising, a nucleotide sequence which encodes an Aiolos polypeptide.
- 20 5. A vector comprising a DNA sequence encoding an Aiolos peptide.
6. A cell containing the purified DNA of claim 4.
7. A method for manufacture of an Aiolos peptide comprising culturing the cell
25 of claim 6 in a medium to express said Aiolos peptide.
8. A method of making an Aiolos polypeptide, having at least one biological activity of a naturally occurring Aiolos polypeptide. including altering the sequence, of one or more residues and testing the altered polypeptide for the desired activity.
30
9. A method for treating an animal for a disorder comprising administering a therapeutically-effective amount of an Aiolos polypeptide, a cell selected for the expression of a product of the Aiolos gene, or a nucleic acid encoding an Aiolos peptide to the animal.
35
10. A method for determining if a subject is at risk for a disorder related to mis-expression of the Aiolos gene comprising examining the subject for the expression or structure of the Aiolos gene, non-wildtype structure or expression being indicative of risk.
40

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11. A transgenic animal having an Aiolos transgene.
12. A substantially pure dimer which includes an Aiolos polypeptide and an Ikaros polypeptide.
- 5 13. A method of providing a proliferation-deregulated cell, or a cell which has non-wild type antibody production comprising causing a subject cell to misexpress the Aiolos gene, thereby providing a proliferation-deregulated or antibody overexpressing cell.
- 10 14. A proliferation-deregulated hematopoietic cell which misexpresses Aiolos.
- 15 15. A method of culturing an Aiolos-misexpressing cell having at least one mutant allele at the Aiolos locus comprising introducing the cell into a mammal and culturing the cell.
- 20 16. A method of reconstituting an immune system comprising supplying a recipient mammal, and introducing into the recipient mammal, an immune system component from a donor mammal, which is Aiolos misexpressing.
- 25 17. A reaction mixture including an immune system component, the component including cells which misexpress Aiolos or being from an animal or cell culture which is misexpresses Aiolos or which carries at least one mutant allele at the Aiolos locus, and a target tissue or cell.

1 / 10

1A. MOUSE AIOLOS cDNA SEQUENCE

CACGAGCGCACACCGCTCGGCTCTCCTTGCGACACGCCCTCATCCCCGGTGTT
TCTCAAGTAGACGTCCCGAGACGGTCGCTGAGGCACTGTTTCCACGCGATCA
GGGTTCCTCAGGCTTGACATTCAAAAGTGGGTGCGGAACCCGCGGCACTCGG
AGCGTGCTTTAAAGCGGCCGCCAGCCAGCGCCGCTCTAACCTCGCGCCCCGG
CTGCCGGCGGCTCCCGCCCTGCATCTGCGCCGACGCGACCGAGCGATCCCGG
GGCCTCCCTGCGCCCCGAATCTCCCGCCAGCCGCGCGGGTCCCCACGGCAGC
AGCACGTGGAGCGGCCGCGGAGCCTGAGCGACAGCTGCAGCCCCGCGCGGCC
CGCGGCGACATGGAAGATATACAACCGACTGTGGAGCTGAAAAGCACGGAG
GAGCAGCCTCTGCCACAGAGAGCCCAGACGCTCTGAATGACTACAGCTTGC
CCAAACCTCATGAGATAGAAAACGTGGACAGTAGAGAAGCCCCAGCCAATG
AAGACGAAGATGCAGGAGAAGATTCGATGAAAGTGAAAGATGAATACAGCG
ACAGAGATGAGAACATTATGAAGCCGGAGCCCATGGGAGATGCAGAAGAGA
GTGAAATGCCTTACAGCTATGCAAGAGAATACAGCGACTATGAAAGCATTAA
GCTGGAGAGACACGTGCCCTATGACAACAGCAGACCAACCAAGTGGAAGAT
GAACTGCGACGTGTGCGGGTTATCCTGCATTAGCTTCAACGTCTTGATGGTTC
ATAAGCGAAGCCATACCGGCGAACGCCCGTTCCAGTGTAATCAGTGCGGGGC
ATCTTTTACTCAGAAAGGTAACCTCCTCCGTCATATTAACTGCACACGGGGG
AAAAACCTTTTAAAGTGTACCTCTGCAACTACGCATGCCAAAGGAGAGATGC
GCTCACGGGACACCTTAGGACACATTCTGTGGAGAAAGCGGTACAAGTGTGAG
TTCTGCGGAAGAAGCTACAAGCAGAGAAGCTCCCTGGAGGAGCACAAAGGAA
CGCTGCCGAGCTTTTCTTCAGAACCCTGACCTGGGGGACGCTGCAAGTGTGG
AGGCAAGACACATCAAAGCCGAGATGGGAAGTGAGAGAGCTCTCGTCCTGG
ACAGATTAGCAAGCAATGTGGCTAAGCGAAAAAGCTCGATGCCTCAGAAATT
CATCGGTGAGAAGCGGCACTGCTTCGATGCCAACTACAATCCCGGCTACATG
TACGAGAAGGAGAACGAGATGATGCAGACCCGGATGATGGACCAAGCCATC
AATAACGCCATCAGCTATCTAGGGGCTGAAGCCTTCCGCCCCTTAGTCCAGA
CTCCGCCTGCTCCACCTCTGAGATGGTCCCAGTCATCAGCAGTGTGTACCCC
ATAGCACTTACTCGGGCCGATATGCCAATGGGGGCCCCGCAgGAGATGGAAA
AGAAACGGATCCTCCTGCCAGAGAAGATCTTGCCTTCTGAACGAGGTCTGTC
CCCCAATAACAGTGCCCAAGGACTCCACAGACACCGACAGCAACCACGAGGAT
CGCCAACATCTCTACCAGCAAAGCCACGTGGTCCTCCCCCAGGCCCGCAATG
GGATGCCTCTTCTGAAGGAGGTCCCTCGCTCTTTTGAACCTCTCAAGCCCCCT
CCCATCTGCCTGAGGGACTCCATCAAAGTGATCAACAAAGAAGGGGAGGTGA
TGGATGTGTTTCGATGTGACCACTGCCACGTCTCTTCTCCTAGATTATGTGATG
TTCACCATCCACATGGGGTGCCATGGTTTCCGTGATCCCTTTGAGTGTAACAT
GTGTGGCTATCGAAGCCACGATCGCTATGAGTTCTCCTCTCACATCGCCAGAG
GAGAGCACAGAGCCATGTTGAAGTGAGCATCTGTCCTCAATGCGAGGGTCAA
CATTGTTTTTTAAAGCTGATGGTAGCCTTATCCAGTAGACTGAACTCAAACCC
ACCTCGAG

FIG. 1

SUBSTITUTE SHEET (RULE 26)

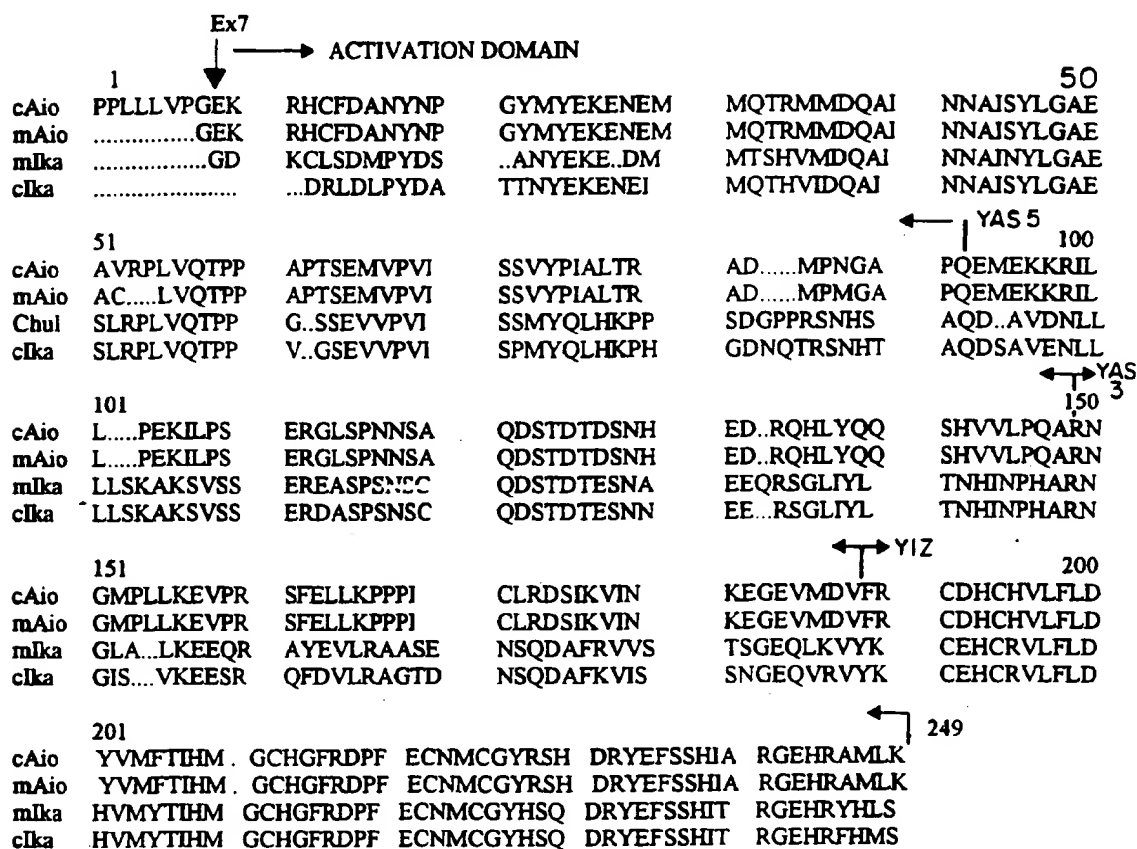
2/10

1B. MOUSE AIOLOS PEPTIDE SEQUENCE

MEDIQPTVELKSTEEQLPTESPDALNDYSLPKPHEIENVDSREAPANEDAGED
SMKVKDEYSRDNIMKPEPMGDAEESEMPYSYAREYSDYESIKLERHVPYDNS
RPTSGKMNCDCGLSCISFNVL MVHKRSHTGERPFQCNQCGASFTQKGNLLRHI
KLHTGEKPFKCHLCNYACQRRDALTGHLRTHSVEKPYKCEFCGRSYKQRSSLEE
HKERCRAFLQNPDLGDAASVEARHIKAEMGSERALVLDRLASNVAKRKSSMPQ
KFIGEKRHCFDANYNPGYMYEKENEMMQTRMMDQAINNAIS'ILGAEAFRPLVQ
TPPAPTSEMVPVISSVYPALTRADMPMGAPQEMEKKRILLPEKILPSEGLSPNN
SAQDSTD TDSNHEDRQHLYQQSHVLPQARNGMPLLKEVPRSFELLKPPPICLRD
SIKVINKEGEVMDVFRCDHCHVFLDYVMFTIHMGCCHGFRDPFECNMCgyrsh
DRYEFSSHIARGEHRAMLK

FIG. 1
(CONT)

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YAS 5 = INTERACTION DOMAIN

YAS 3 = INTERACTION DOMAIN

YIZ = IKAROS DIMERIZATION DOMAIN

FIG. 2**SUBSTITUTE SHEET (RULE 26)**

				50
aio
Ik1	MDVDEGQDMS	QVSGKESPPV	SDTPDEGDEP	MPVPEDLSTT
				SGAQQNSKSD
51				
aio
Ik1	RGMASNVKVE	TQSDEENGRA	CEMNGEECAE	DLRMLDASGE
				KMNGSHRDQG
				E x 4
				↓
101				150
Ik	NSARGKMNCD	VCGLSCISFN	GERPFQCNQC
Ik1	SSALSGVGGI	RLPNGKCLKCD	ICGIVCIGPN	GERPFQCNQC
				E x 5
				↓
151				200
aio	GASFTQKGNL	LRHIKLHTGE	KPFKCHLCNY	ACQRRDALTG
Ik1	GASFTQKGNL	LRHIKLHSGE	KPFKCHLCNY	ACRRRDALTG
				E x 6
				↓
201				250
Aio	YKCEFCGRSY	KQRSSLEEHLK	ERCRAFLQNP	DLGDAASV.
Ik1	HKCGYCGRSY	KQRSSLEEHLK	ERCHNYLESM	GLPGMYPVIK
				E x 7
				↓
251				300
Aio	IKAEMGSERA	LVLDRLASNV	AKRKSSMPQK	FIGEKRHCDFD
Ik1	DLCKIGAERS	LVLDRLASNV	AKRKSSMPQK	FLGDK. .CLS
				ANYNPGYMYE
				DMPYDSANYE
301				350
Aio	KENEMMQTRM	MDQ.
Ik1	KE. DMMTSHV	MDQ		

FIG. 3

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EXON 3	IRHEEAPANEDEDAGEDSMK VKDEYSDRDENIMKPEPMGDAEESEMPYSYA REYSDYESIKLERHVPYDNSRPTSGKMNCDCGLSCISFNVLMVHKRSHT
EXON 4	GERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNYACQRRDALTGH LRTHS
EXON 5	VEKPYKCEFCGRSYKQRSSLEEHHKERCRAFLQNPDLGDA
EXON 6	ASVEARHIKAEMGSERALVLDRLASNVAKRKSSMPQKFI
EXON 7	GEKRHCFDANYNPGYMYEKENEMMQTRMMDQAINNAISYLGAEAFRLVQ TPAPTSEMVPVISSVYPIALTRADMPMGAPQEMEKKRILLPEKILPSERG LSPNNSAQDSTTDSNHEDRQHL YQQSHVVL PQARNGMPLLKEVPRSFEL LKPPPICLRDSIKVINKEGEVMDVFRCDHCHVLFLDYVMFTIHMGGCHGFRD PFECNMCGYRSHDRYEFSSHIARGEHRAMLK

3	4	5	6	7
3	4	5	7	
ALT	7			

FIG. 4

10 20 30 40 50 60 70 80 90
 GAAAGAGATGACAAATGTTTTAAAGTCAGAAACCAATGGGAAATGCAGAGAGCCTGAAATCCCTTACAGCTATTCAAGAGAGATATATATGAA 90
 TATGATAAACAATTAAAGTTGGAGAGACATGTTGTCTCATTCGTAGTAGCAGGCCAACACGATGGGAAAGATGAACTGGCATGTGTGTGGATTGA 180
 TCCTGCATCAGCTTCAATGTCCTTAATGGTTTCATTAAGCGAAGCCATACtGtGaaagccattccagtgtaatcagtggtgaggtggtggtcattctt 270
 actcagaagaagttaacctcctccGccacattaaAcbtgcacacaggggaaabaaacctttttaagtgtcacctctgcaactatgcatgcccagaaga 360
 agagatggctcaaggggcattcttaggacacattctgtggagaaacccatacaaatgtgagtttttgtggaaggaggttacaagcagagagaagt 450
 460 470 480 490 500 510 520 530 540
 tcccttgaggagcacaaggagcgtgocgtacattcttcagagcaactgaccabgggacacCTcAAGTGGGAGGCGAAGACACACTCAAA 540
 CCACAGATGGGAAAGTGAAGAGAGCTCTGTACTGGACAGATTAGCAAGCAATGIGTCAAAAACGAAAAAGCTCAATGCTCTCAGAAATTCA 628

FIG. 5A

Lipman-Pearson Protein Alignment									
kTuple: 2; Gap Penalty: 4; Gap Length Penalty: 12		Seq2(1>508)		Similarity		Gap		Consensus	
Seq1(1>209)		mousealolos.protein		Index		Number		Length	
human Aiolos protein AioC/hAio2		(66>273)		89.5		1		209	
human Aiolos protein AioC/hAio2	ERDENVLKSEPHMGNAEEPI	PYSYSREYNEYENIKLERHVSF	DSSRPTSGKMNCDVCG	60					
mousealolos.protein	: RDEN: : K: EPMG: AEE: E: PYSY: REY: : YE: IKLERHV : : D: SRPTSGKMNCDVCG								
	DRDENIMKPEPMGDAE	SEMPYSYAREYSDYESIKLERHV	-PYDNSRPTSGKMNCDVCG	124					
human Aiolos protein AioC/hAio2	SCISFNVLNVHVKRSHTGER	PFQCNQCGASFTQKGNLLRH	IKLHTGEKPFKCHLCNYACQR	120					
mousealolos.protein	SCISFNVLNVHVKRSHTGER	PFQCNQCGASFTQKGNLLRH	IKLHTGEKPFKCHLCNYACQR	184					
	SCISFNVLNVHVKRSHTGER	PFQCNQCGASFTQKGNLLRH	IKLHTGEKPFKCHLCNYACQR	184					
human Aiolos protein AioC/hAio2	RDALTGHLRTHSVEKPYKCE	FCGRSYKORSSLEE	HKERCRTFLOSTDPGDTASAEARHIK	180					
mousealolos.protein	RDALTGHLRTHSVEKPYKCE	FCGRSYKORSSLEE	HKERCRTFLOSTDPGDTASAEARHIK	244					
	RDALTGHLRTHSVEKPYKCE	FCGRSYKORSSLEE	HKERCRTFLOSTDPGDTASAEARHIK	244					
human Aiolos protein AioC/hAio2	AEMGSERALVLDRLASNVAKRKSSMPQKF		209						
mousealolos.protein	AEMGSERALVLDRLASNVAKRKSSMPQKF		273						
	AEMGSERALVLDRLASNVAKRKSSMPQKF		273						

7/10

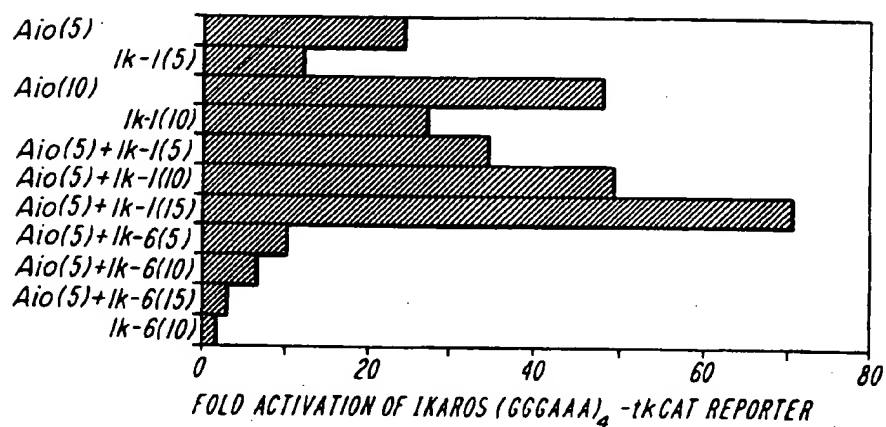
FIG. 5B

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[illegible]

FIG. 6
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**FIG. 7**

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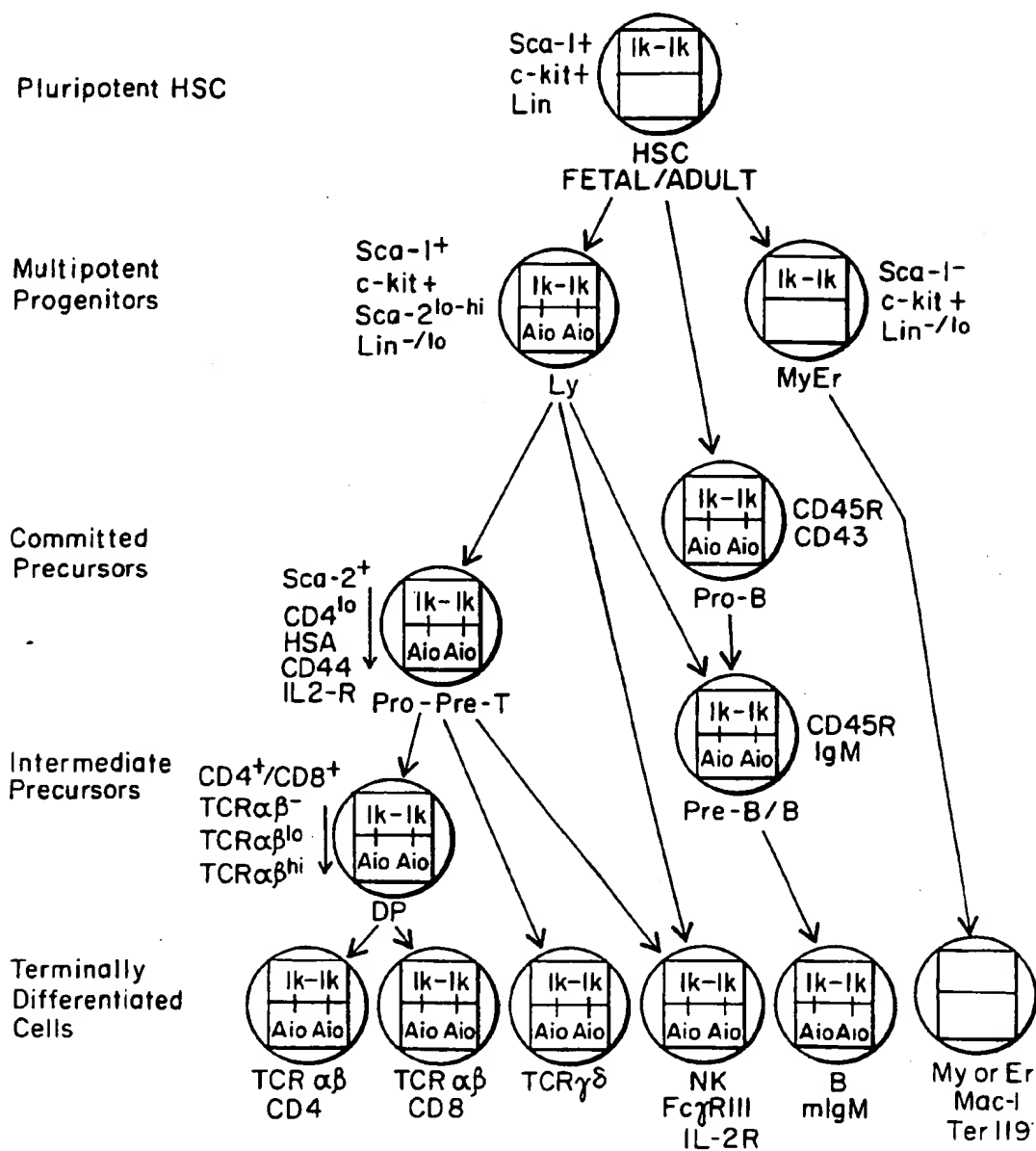


FIG. 8

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/16774

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, GENBANK, REGISTRY, CAPLUS, BIOSIS, WPIDS, EMBASE

search terms: Aiolos, Ikaros, Georgopoulos, K, Dimers, Lymphocyte, zinc finger, 7peptide,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	WINANDY et al. A dominant mutation in the Ikaros gene leads to rapid development of leukemia and lymphoma. Cell. 20 October 1995, Vol. 83, No. 2, pages 289-299, see entire document.	1-17
A	HAHM et al. The lymphoid transcription factor LyF-1 is encoded by specific, alternatively spliced mRNAs derived from the Ikaros gene. Molecular and Cellular Biology. November 1994, Vol. 14, No. 11, pages 7111-7123, see entire document.	1-17
A	WO 94/06814 A1 (THE GENERAL HOSPITAL CORPORATION) 31 March 1994, see entire document.	1-17

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
O documents referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

17 DECEMBER 1996

Date of mailing of the international search report

03 FEB 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DEBORAH CLARK

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16774

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GEORGOPOULOS et al. The Ikaros gene is required for the development of all lymphoid lineages. Cell. 07 October 1994, Vol. 79, No. 1, pages 143-156, see entire document.	1-17
A	MOLNAR et al. The Ikaros gene encodes a family of functionally diverse zinc finger DNA-binding proteins. Molecular and Cellular Biology. December 1994, Vol. 14, No. 12, pages 8292-8303, see entire document.	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16774

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 2
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The claim is drawn to fragments of the Aiolos peptide which is at least 50 amino acids in length. The sequence rules were not complied with and therefore no sequence search could be conducted. All other claims were searched by keywords.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Intern. application No.

PCT/US96/16774

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07K 14/435, 16/18; A61K 48/00, 49/00, 39/395; C07H 21/04; C12N 15/85, 15/00, 15/63; C12P 21/00; G01N 33/53; A01K 67/00;

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

530/350, 388.85, 389.1; 424/130.1, 93.1; 536/23.1; 435/320.1, 240.2, 70.1, 69.1, 7.1; 514/44; 800/2;

B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

530/350, 300; 424/130.1, 93.1, 9.1; 536/23.1, 24.5; 435/320.1, 240.2, 240.1, 70.1, 69.1, 7.1, 70.3; 514/44, 2; 800/2, DIG 1, DIG 2;

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/05097

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C12N 15/00; C12Q 1/68

US CL : 435/6, 172.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 172.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Developmental Biology, Volume 101, issued 1984, K. H. Cox et al, "Detection of mRNAs in Sea Urchin Embryos by <u>in situ</u> Hybridization using Asymmetric RNA Probes", pages 485-502, entire document.	1-9,11-20,20,30,43 and 47-49
X	R. B. Church, "Transgenic Models in Medicine and Agriculture" published 1990 by Wiley-Liss, Inc., pages 33-45.	10 and 21-50

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance		
"E" earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search

11 August 1992

Date of mailing of the international search report

27 AUG 1992

Name and mailing address of the ISA/
Commissioner of Patents and Trademarks
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